

# The effects of prenatal and neonatal exposure to electromagnetic fields on infant rat myocardium

Hamid Tayefi<sup>1</sup>, Amac Kiray<sup>1</sup>, Muge Kiray<sup>2</sup>, Bekir Ugur Ergur<sup>2</sup>, Husnu Alper Bagriyanik<sup>2</sup>, Cetin Pekcetin<sup>2</sup>, Mustafa Fidan<sup>3</sup>, Candan Ozogul<sup>2</sup>

<sup>1</sup>Departments of Anatomy, Dokuz Eylul University Medical School, Balçova, Izmir, Turkey

<sup>2</sup>Departments of Histology & Embryology, Dokuz Eylul University Medical School, Balçova, Izmir, Turkey

<sup>3</sup>Departments of Biophysics, Dokuz Eylul University Medical School, Balçova, Izmir, Turkey

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**Corresponding author:**

Muge Kiray, MD, PhD

Department of Histology

& Embryology

Dokuz Eylul University

Medical School

Balçova, Izmir 35340, Turkey

Phone: +90 232 412 45 62

Fax: +90 232 259 05 41

E-mail:

muge.kiray@deu.edu.tr

## Abstract

**Introduction:** Electromagnetic fields (EMF) have adverse effects as a result of widespread use of electromagnetic energy on biological systems. The aim of this study was to investigate the effects of prenatal exposure to EMF on rat myocardium by biochemical and histopathological evaluations.

**Material and methods:** In this study, 10 pregnant Wistar rats were used. Half of the pregnant rats were exposed to EMF of 3 mT, and the other half to sham conditions during gestation. After parturition, rat pups in the 5 EMF-exposed litters from birth until postnatal day 20 were exposed to EMF of 3 mT for 4 h/day (EMF-exposed group,  $n = 30$ ). Rat pups in sham litters from birth until postnatal day 20 were exposed to sham conditions (sham group,  $n = 20$ ).

**Results:** In the EMF-exposed group, lipid peroxidation levels significantly increased compared to sham. Superoxide dismutase activities decreased significantly in the EMF-exposed group compared to sham. TUNEL staining showed that the number of TUNEL-positive cells increased significantly in EMF-exposed rats compared with sham. Under electron microscopy, there were mitochondrial degeneration, reduction in myofibrils, dilated sarcoplasmic reticulum and perinuclear vacuolization in EMF-exposed rats.

**Conclusions:** In conclusion, the results show that prenatal exposure to EMF causes oxidative stress, apoptosis and morphological pathology in myocardium of rat pups. The results of our study indicate a probable role of free radicals in the adverse effects of prenatal exposure to EMF. Further studies are needed to demonstrate whether the EMF exposure can induce adverse effects on the myocardium.

**Key words:** electromagnetic field, myocardium, oxidative stress, apoptosis, ultrastructure

## Introduction

Electromagnetic fields (EMF) have adverse effects on biological systems. Due to advances in technology and widespread use of electromagnetic energy, today everyone is exposed to EMF. 50-60 Hz EMF are generated from domestic electric distribution systems. EMF affect biological systems by prolonging the life of free radicals in the systems. Free oxygen radicals cause oxidative damage to DNA, impairment of protein function and peroxidation of lipids. Malondialdehyde (MDA) is a breakdown product of the major chain

reactions leading to the oxidation of polyunsaturated fatty acids and thus causing oxidative stress [1]. EMF could cause oxidative DNA damage and increase lipid peroxidation in tissues [2, 3]. Reports have demonstrated that oxidative damage has been implicated in myocardial injuries [4]. Ozguner *et al.* reported an increase in lipid peroxidation in rat heart following exposure to EMF [5]. Antioxidant defences and repair systems which help to protect cells against free radical destruction include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) [6, 7]. There are several reports which indicate that exposure to EMF reduces antioxidant enzyme activities in rat tissues [8, 9].

The cellular damage induced by oxidative stress may trigger the process of apoptosis. Free oxygen radicals may participate in the initiation of apoptotic or necrotic cell death. EMF can induce apoptosis *in vivo* and *in vitro* [10-12].

Several structural changes may occur after myocardial damage. Myocardial changes could be revealed by light or electron microscopic evaluation. The aim of this study was to investigate the effects of prenatal exposure to EMF on rat hearts by biochemical and histopathological evaluations. For biochemical evaluation, MDA levels were assessed as a marker of lipid peroxidation and the activities of SOD were determined to evaluate antioxidant status in the rat hearts. Light and electron microscopic assessment was carried out for histopathological evaluation.

## Materials and methods

### Animals and experimental design

All experimental protocols were approved by the Ethics Committee of Animal Care and Experimentation of the University of Dokuz Eylul, Turkey. In this study, 10 pregnant Wistar rats were used for prenatal exposure of EMF. They were housed in individual polycarbonate cages with food and water *ad libitum*. Half of the pregnant rats were exposed to EMF of 3 mT for 4 h a day, and the other half were separated for a sham group during gestation [13]. After parturition, rat pups in the 5 EMF-exposed litters from birth until postnatal day 20 were exposed to EMF of 3 mT for 4 h/day, 7 days/week (EMF-exposed group,  $n = 30$ ). Rat pups in sham litters from birth until postnatal day 20 were exposed to sham conditions (sham group,  $n = 20$ ). At 21 days of age, rat pups were separated from maternal rats, sacrificed and evaluated, without regard to their sex [14].

### Magnetic field exposure system

EMF of 3 mT was produced by a pair of Helmholtz coils (95 cm in diameter) with 320 turns of 2.5 mm copper wire in each, mounted on a wooden frame. The distance between coils was

33 cm. Coils were connected in series to a generator delivering an AC current. The output current was 6.43 A at 50 Hz. The magnetic field intensity was measured by a digital teslameter (FW Bell, 5170). The teslameter accuracy was  $\pm 2\%$  for AC.

### Biochemical estimations

The animals were sacrificed 24 hours after the last exposure, and heart tissue samples were taken out for light and electron microscopic assessment and for biochemical estimations. Determination of MDA levels and antioxidant enzyme activity were performed spectrophotometrically. The Bioxytech MDA-586 (Oxis International, USA) assay for MDA and the Bioxytech SD-525 (Oxis International, USA) assay for SOD activity were performed as per the kit protocol. All enzyme activities were assayed with a Hach Lange DR5000 UV spectrophotometer.

### Histopathological examination

The dissected rat hearts were immediately placed in 10% formalin in phosphate buffer overnight, processed by routine histological methods and embedded in paraffin blocks. Paraffin blocks were placed in a Leica RM2125 rotary microtome (Germany) and sections of 5  $\mu\text{m}$  thickness were obtained. Sections were stained for TUNEL. The images were analysed using a computer-assisted image analyser system consisting of a microscope (Olympus BH-2 Tokyo, Japan) equipped with a high-resolution video camera (JVC TK-890E, Japan).

For ultrastructural investigations, the left ventricle pieces were placed in 2.5% glutaraldehyde for 24 hours for fixation. The tissue was postfixed with osmium tetroxide ( $\text{OsO}_4$ ), dehydrated in a graded series of alcohol, and then embedded in Araldite® CY212. Thin (60–90 nm) sections were obtained with an ultramicrotome (Leica), stained with uranyl acetate and lead citrate, examined on a transmission electron microscope (Carl Zeiss Libra 120), and digitally photographed.

### Terminal deoxynucleotidyl transferase mediated dUTP nick end labelling (TUNEL) method

TUNEL staining was performed using an In Situ Cell Death Detection Kit® (Roche, Mannheim, Germany) according to the manufacturer's protocol. Briefly, the sections were deparaffinized, hydrated by successive series of alcohol, washed in distilled water followed by phosphate-buffered saline (PBS) and deproteinized by proteinase K (20  $\mu\text{g}/\text{ml}$ ) for 30 min at 37°C. Then the sections were rinsed and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using converter-POD with 0.02% 3,3'-diaminobenzidine (DAB). The sections were counter-stained with haematoxylin.

Ten fields were randomly chosen for each slide and a total of 100 cells per field were counted (X20 objective). The apoptotic index (percentage of apoptotic nuclei) was calculated as apoptotic nuclei/total nuclei counted  $\times$  100%. All counting procedures were performed blindly.

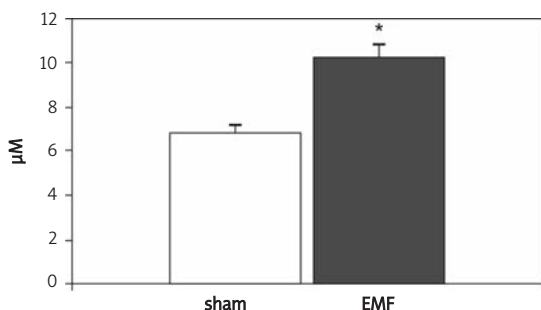
### Statistical analysis

Results are presented as means  $\pm$  SEM. All data were analysed by Mann-Whitney test.  $P < 0.05$  was considered statistically significant.

## Results

### Biochemical analyses

Figure 1 shows the levels of MDA in hearts of rat pups. MDA level in the sham group was  $6.84 \pm 0.27$ .

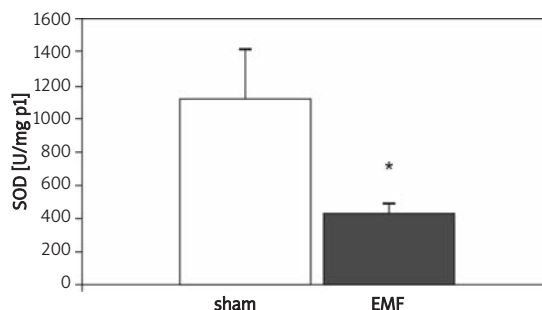


**Figure 1.** Effects of prenatal exposure to EMF on MDA levels in heart of rat pups. Data are mean  $\pm$  SEM  
\* $P < 0.05$  compared with the sham group

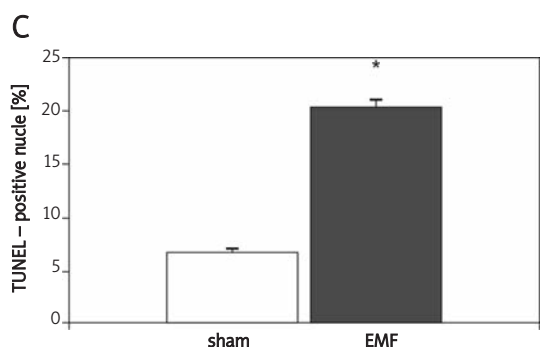
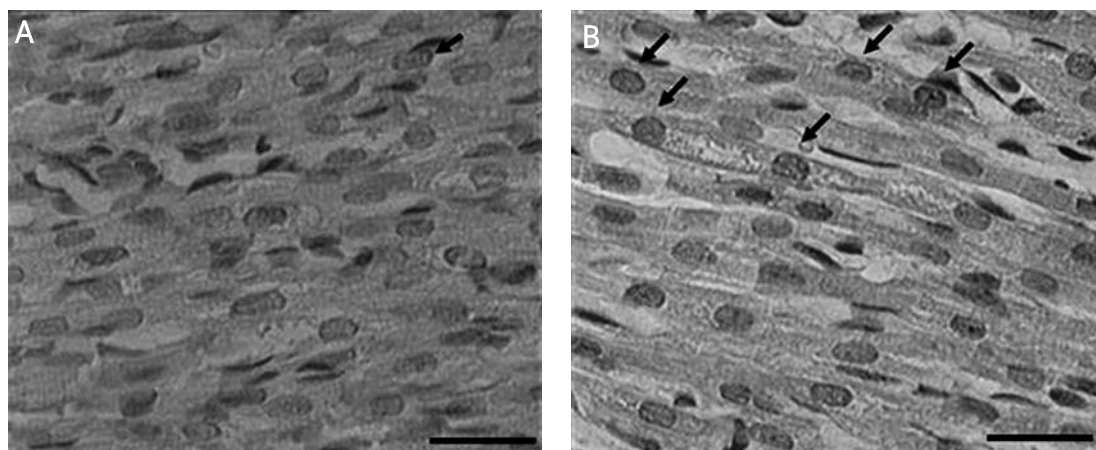
In the EMF-exposed group, tissue MDA levels were significantly increased compared to the sham group ( $10.21 \pm 0.63$ ,  $p < 0.05$ ). The results of SOD enzyme activities are shown in Figure 2. SOD enzyme activities decreased significantly in the EMF-exposed group compared to the sham group ( $425.3 \pm 65.6$  and  $1125 \pm 288.5$ , respectively).

### Histopathological examination

The present study shows that EMF exposure enhanced apoptotic cell death in the rat myocardium. Sham rats showed fewer TUNEL-positive cells in the myocardium. There were more TUNEL-positive cells in the EMF-exposed group, as seen in Figure 3. Quantification and statistical analysis of the TUNEL staining showed that the



**Figure 2.** Effects of prenatal exposure to EMF on SOD activity in heart of rat pups. Data are mean  $\pm$  SEM  
\* $P < 0.05$  compared with the sham group



**Figure 3.** Upper: The effect of prenatal exposure to EMF on apoptosis in heart of rat pups. Representative photomicrographs of TUNEL-positive cells (arrows). **A** and **B** show sections of sham and EMF groups, respectively (scale bar = 30  $\mu$ m). Lower: Quantitative analysis of TUNEL-positive cells in the rat heart. Data are mean  $\pm$  SEM  
\* $P < 0.05$  compared with the sham group

number of TUNEL-positive cells increased significantly in EMF-exposed rats compared with sham rats ( $p < 0.05$ ).

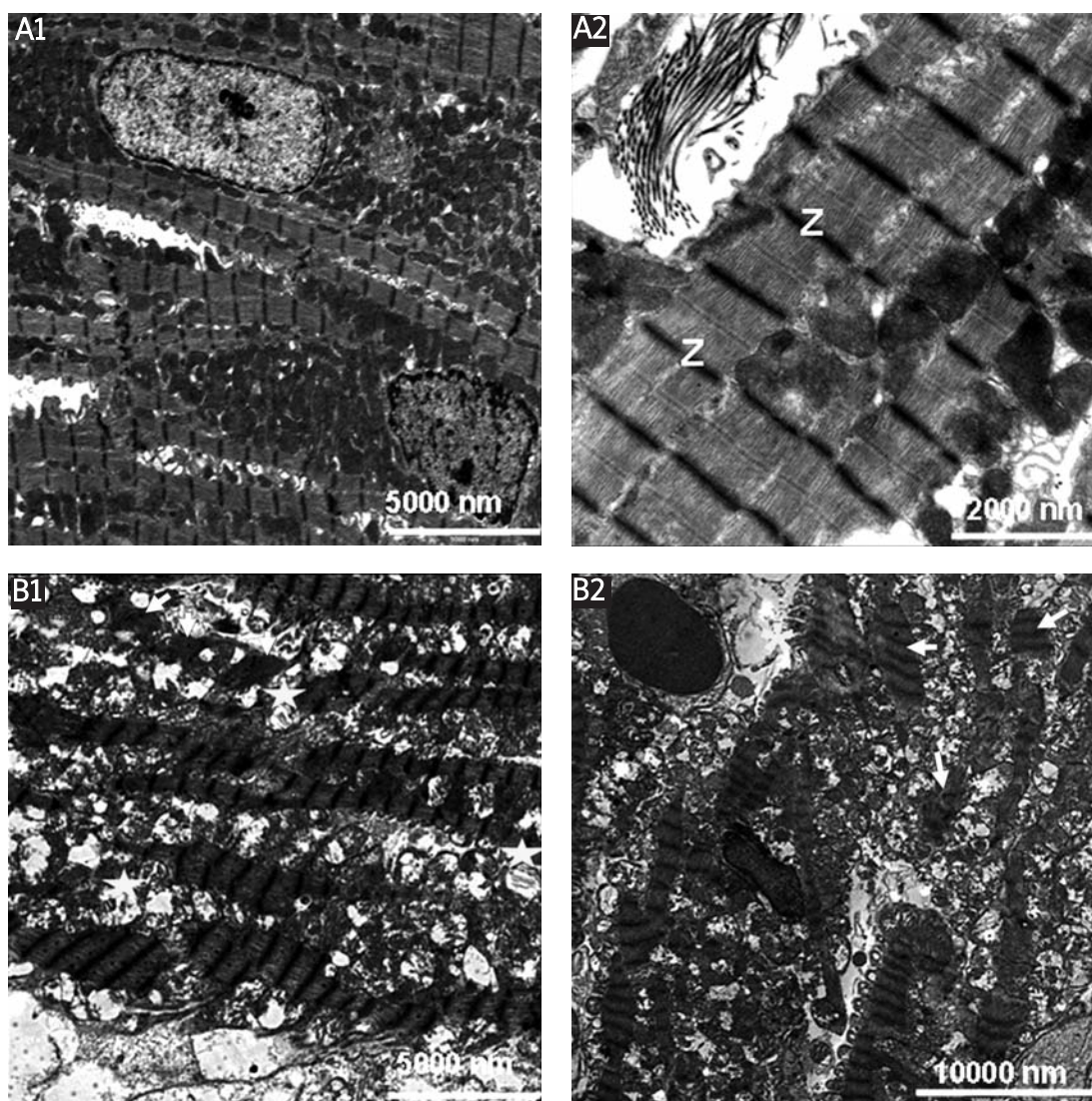
In electron microscopic examination, no pathological changes were observed in heart specimens of the sham group; cellular and mitochondrial structures were normal. In EMF-exposed group specimens, there were mitochondrial degeneration and dilated sarcoplasmic reticulum. Most myofibrils had disappeared or were fragmented and perivascular vacuoles had formed (Figure 4).

### Discussion

In this study, we demonstrated that EMF causes oxidative damage by increasing MDA levels and suppressing SOD activities in heart tissue in a rat

model of prenatal exposure to EMF. Additionally, the findings of this study indicated that EMF exposure induced apoptosis and ultrastructural changes in heart tissue.

Our results indicate that exposure to 3 mT EMF induces lipid peroxidation in myocardial tissue of rat pups. Lipid peroxidation is one of the determinants of free radical induced oxidative damage. Experimental studies suggest that lipid peroxidation resulting from free oxygen radicals contributes to the EMF-induced oxidative damage [15, 16]. The organism has some protective defence enzymes and repair systems against oxidative damage. In this study, we assessed SOD activities in myocardial tissue of rat pups. The antioxidant enzyme activities were found to be significantly decreased in EMF-exposed rats. Our findings support those of other investigators who



**Figure 4.** Electron micrographs of the left ventricular myocardium of sham (A1, A2) and EMF-exposed groups (B1, B2). A1-A2, normal myocytes, mitochondria and Z-lines (Z). B1, degeneration and fragmentation of myofibrils (arrows), loss of cristae and swelling in mitochondria (\*). B2, fragmentation of myofibrils (arrows) and perivascular vacuoles (\*)

demonstrated an increase in lipid peroxidation and decrease in antioxidant enzyme activities in heart tissue with EMF exposure, while the effect of prenatal exposure to EMF in heart tissue has not been well established yet [5].

At the end of the experiment the cardiomyocyte apoptosis was detected by the TUNEL method. Our results showed that prenatal exposure to EMF induces apoptotic cell death in rat pups. The effect of EMF on cardiomyocyte apoptosis is associated with marked increase in MDA. The mechanism through which 3 mT EMF can affect cell death may be due to free radicals. Cardiomyocyte apoptosis is associated with several cardiac diseases. Although increased apoptotic activity in heart tissue with EMF exposure has been reported in vitro, the effect of prenatal exposure to EMF in rat pups has not been previously investigated [17].

In this study histological evidence of EMF-related myocardial tissue injury was shown with electron microscopy. The ultrastructural modifications were assessed to investigate the adverse effects of prenatal exposure to EMF in heart tissue. Under electron microscopy, mitochondrial swelling, reduction in myofibrils, expanded sarcoplasmic reticulum and perinuclear vacuolization were observed. The pathological alterations in myocardial ultrastructure have been shown in various myocardial damage models. Yaras *et al.* demonstrated that diabetic rat hearts show mitochondrial and myofibrillar degeneration. Bartel *et al.* reported pathological changes in sarcoplasmic reticulum, mitochondria and myofibrils in drug cardiotoxicity [18, 19]. In our study, we demonstrated firstly prenatally EMF-induced myocardial pathological changes in ultrastructure of rat pups.

In conclusion, the results show that prenatal exposure to EMF causes apoptosis and morphological pathological changes in myocardium of rat pups. EMF exposure induces oxidative stress through reducing SOD activity parallel to increasing lipid peroxidation. The results of our study indicate a probable role of free radicals in the adverse effects of prenatal exposure to EMF in myocardium of rat pups. Although EMF has been studied for developmental and memory abnormalities in rats, further studies are needed to demonstrate whether EMF exposure can induce adverse effects on rat myocardium.

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