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The effect of extremely low frequency magnetic field on heart tissue iron density

Aşırı düşük frekanslı manyetik alanın kalp dokusu demir yoğunluğu üzerine etkisi

Sevda Söker[1], Cemil Sert[2], Mustafa Deniz[3], Ercan Ayaz[1], Selçuk Tunik[1],Yusuf Nergiz[1]

[1]*Medicine Faculty of Dicle University, Department of Histology and Embryology, Diyarbakır, Turkey*
[2]*Department of Biophysics, Medicine Faculty of Harran University, Şanlıurfa, Turkey*
[3]*Department of Anatomy, Medicine Faculty of Harran University, Şanlıurfa, Turkey*

ABSTRACT

Objectives: The aim of this histological study was to investigate the effects of extremely low frequency, low intensity magnetic field on the heart muscle ıron density.

Materials and methods: In this study, 45 male Spraque Dawley rats were introduced and were divided into three groups as sham, control and experiment group. The experimental group was exposed to a 0.25 mT to Extremely Low Frequency Magnetic Field (ELF-MF) for 14 days, 3h a day in metacrylate boxes. The sham group was treated like the experimental group, except for ELF-MF exposure. For control, nothing applied to rats in this group and they completed their life cycle in the cage during the study period. After exposure period, the rats were sacrificed under ketalar anesthesia (50 mg / kg, intramuscularly). Heart tissues were immediately fixed in 10% neutral formaldehyde and embedded in paraffin blocks. Histological sections from cardiac tissue stained by hematoxylin-eosin, Perls' Prussian blue for iron pigments. Histological slides were photographed under a Nikon DS-2MV photomicroscope.

Results: The architecture and histology of the control, sham and experimental group were observed as normal. No differences were observed between the control, sham and experimental rat groups in the iron stain of heart tissues.

Conclusion: As a result of our study, we did not observe differences between the control and ELF-MF (experimental) group. In this investigation we demonstrated that the exposure of cardiac tissue of rats to the ELF-MF did not change in the iron stain study. *J Clin Exp Invest 2011;2(2):144-8*

Key words: Extremely low frequency magnetic field, heart, iron.

ÖZET

Amaç: Bu histolojik çalışmanın amacı; aşırı düşük frekanslı, düşük yoğunluklu manyetik alanın kalp dokusu demir yoğunluğu üzerindeki etkilerini araştırmaktır.

Gereç ve yöntem: Bu çalışmada; sham, kontrol ve deney grubu olarak üçe bölünen 45 Spraque Dawley erkek rat kullanıldı. Deney grubu günde üç saat 14 gün boyunca metakrilat kutularda 0.25 mT oldukça düşük frekanslı manyetik alana (ELF-MF) maruz bırakıldı. Sham grubu, ELF-MF uygulaması dışında deney grubuna benzer şekilde uygulamaya maruz bırakıldı. Kontrol grubu ratlara hiçbir şey uygulanmadı ve fareler çalışma peryodu boyunca yaşam sikluslarını kafes içinde tamamladı. Yapılan işlem sonrasında, 50 mg/kg intramuskuler ketalar anestezisi uygulanarak ratlar sakrifiye edildi Kalp dokuları hemen %10 nötral formalinde fikse edilerek parafin bloklara gömüldü. Kalp dokusunun histolojik örnekleri Hematoksilen-Eosin ve demir pigmentleri için Perls' Prussian blue boyası ile boyandı. Nikon DS-2MV fotomikroskopta histolojik preparatlar fotoğraflandı.

Bulgular: Kontrol, sham ve deney grubumuzun histolojik yapısı normal gözlendi. Kontrol, sham ve deney grubu arasında kalp dokusu demir boyaması açısından farklılık gözlenmedi.

Sonuç: Çalışmamız sonucunda, kontrol ve ELF-MF (deney) grubu arasında farklılık saptamadık. Bu çalışmada, ELF-MF'ye maruz kalan farelerin kalp dokusunda demir boyaması ile değişiklik olmadığı saptandı. *Klin Deney Ar Derg 2011;2(2):144-8*

Anahtar Kelimeler: Aşırı düşük frekanslı elektromanyetik alan, kalp, demir.

Yazışma Adresi /Correspondence: Sevda Söker, MD, PhD, Dept. Histology and Embryology, Medicine Faculty of Dicle University, Diyarbakır, Turkey, Email: ipeksoker@hotmail.com
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INTRODUCTION

In modern societies, humans are frequently exposed to magnetic fields, including extremely low frequency, low intensity and high intensity magnetic fields. Low intensity magnetic fields are generally produced by power lines and many kinds of electrical appliances. High intensity magnetic fields are produced by the apparatus such as magnetic resonance imaging equipment. An increased health risk due to exposure to electromagnetic fields (EMF) at 50 and 60 Hz has been reported by several authors.[1-4]

Iron is an essential element in a variety of vital processes, including respiratory electron transfer, oxygen transport.[5] However, when iron reacts with H_2O_2, hydroxyl radicals are produced via a Fenton-type reaction. The hydroxyl radical is the most powerful oxidizing species among several reactive-oxygen radicals, and is capable of oxidizing most macromolecules including nucleic acids, lipids, and proteins. Ferritin is the protein responsible for iron storage.[6] Cellular uptake of circulating excess iron results in increased formation of ferritin and hemosiderin found in highest concentrations in parenchymal tissue of several organs. As long as iron is bound to ferritin, cytotoxic reactions are not expected to occur. When iron is released from ferritin, low molecular iron complexes may undergo redox reactions, thus inflicting cytotoxic damage upon macromolecules.[7-8] Iron ions are strong catalysts for the peroxidation of membrane lipids, and give rise to membrane damage.[7,9,10]

Electromagnetic fields have adverse effects as a result of widespread use of electromagnetic energy on biological systems. Several experimental and biological studies have dealt especially with increased incidence of various types of cancer, including childhood leukemia, lymphomas, brain tumours and breast cancers, effects on reproduction and development and behavioral changes.[1,11] In vivo and in vitro investigations claim that extremely low-frequency magnetic field produced a genotoxic effect, originating from types of free radicals.[12,13] For biological effects of free radicals, especially reactive oxygen species (ROS) may produce cellular and toxic effects such as lipid peroxidation in cell membrane, protein degradation, enzyme inactivation.[12,13] Increased iron content of cells and tissue may increase the risk of cancer. In particular, high available iron status may increase the risk of a radiation-induced cancer. Iron can catalyze the production of oxygen radicals.

Among the possible health effects of exposure to extremely low frequency magnetic fields, cardiovasculer effects have been reported in the database of EMF bioeffects.[14, 15] Recent studies have investigated whether exposure to a magnetic field poses a risk for cardiovascular morbidity and mortality.[16, 17] A statistically significant relationship was found between exposure to a magnetic field and reduced heart rate variability, which leads to certain disorders such as acute myocardial infarctus and cardiac arrhythmia.[18] The effects of ELF-MF have been investigated on heart muscle tissue limited number of histological studies. The aim of this study was to investigate the possible effects of ELF-MF on heart, and heart muscle tissue of the intended relationship with iron status.

MATERIALS AND METHODS

Animals

The research was designed and implemented according to the principles of the Declaration of Helsinki. This experimental research was performed with the approval of the ethics committee of Harran University. Spraque Dawley rats were obtained from the Medical Science Application and Research Center of Dicle University.

All animals were 2 months old at the beginning of the study, weighing 250-280 g, and were fed with Standard pellet food (Tavas Inc, Adana, Turkey). The rats were divided three groups of fifteen: Control, sham, and experimental groups. The experimental group was exposed to a 0.25 mT to Extremely Low Frequency magnetic field (ELF-MF) in metacrylate boxes (17x17x25 inches). The experimental group (n=15) was exposed to ELF-MF for 14 days, 3h a day. The sham group was treated like the experimental group, except for ELF-MF exposure. Treatment of the control group differed from the experimental and sham groups. Animals in the control group were kept in a 14/10 hour light/dark environment at constant temperature of 22±10% humidity.

Magnetic field generation and exposure to magnetic field

The magnetic field was generated by a device designed by the researchers that had two pairs of

Helmholtz coils of 25 cm diameter. The frequency of sinusoidal current was 50 Hz. This magnet was constructed by winding 300 turns of insulated soft copper wire with a diameter of 0.85 mm. Coils were placed vertically and horizontally, facing one another. The distance between coils was 25 cm. The average MF intensity was measured as 0.25 mT ± 0.01 mT at 12 different points both transverse and axial within the metacarylate cage by using a digital hall effect Gauss meter (Bell 5170, SYPRIS, USA). The measurements were made by an independent researcher who was not involved in the animal experiment. No temperature difference was observed between exposure and sham coils during the exposure.

Histological examinations

The animals were euthanized after the final exposure by anesthesia with ketalar (50 mg/kg, intramuscularly). In the end of experiment, rats were sacrificed; cardiac tissue was fixed by 10% neutral formaldehyde and embedded in paraffin blocks. Histological sections from cardiac tissue stained by Hematoxylin-Eosin, Perls' Prussian blue for iron pigments. Nikon DS-2MV camera and NIS elements 3.00 software were used for observation and documentation.

RESULTS

In the end of this study was to investigate the effect of electromagnetic field on heart muscle. It is commonly believed that more than a few ferritin particles in cell cytoplasm is a predictor of iron overload. Histological sections were stained by Perl's Prussian blue method for the observation of " hemosiderin granules". Pearl's Prussian blue (ferrocyanide method) staining made Fe^{+3} apparent. Histological examination in all three groups was carried out by light microscope. In this investigation we demonstrated that the exposure of cardiac tissue of rats to the ELF-MF did not change in the light microscopic study.

The control group and other groups in the architecture and histology were observed as normal (Figure 1, 2, 3). No differences between control, sham and experimental groups rat have been observed in the iron stain of heart tissues (Figure 4,5,6).

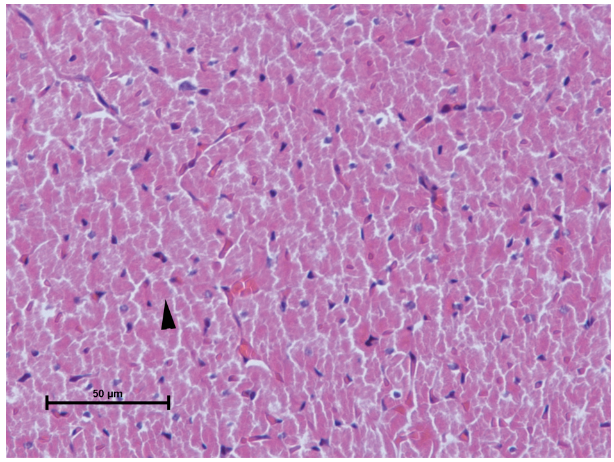

Figure 1. Photo micrograph of Experimental (Exposed to magnetic field) group. Note: Myofibrils (arrowhead) are seen (Hematoxylin-eosin staining, original magnification X 40).

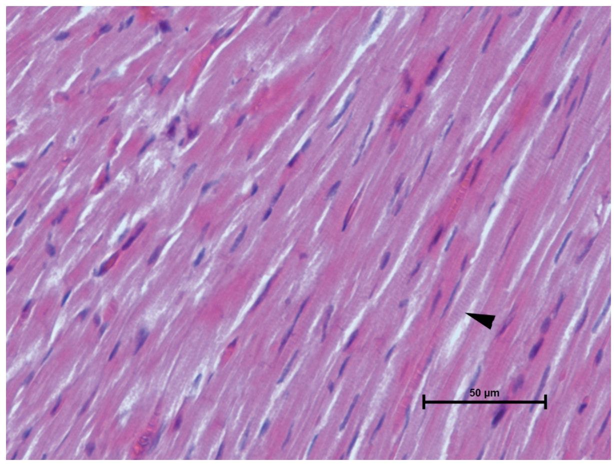

Figure 2. Photo micrograph of Sham group. Note: Myofibrils (arrowhead) are seen (Hematoxylin-eosin staining, original magnification X 40).

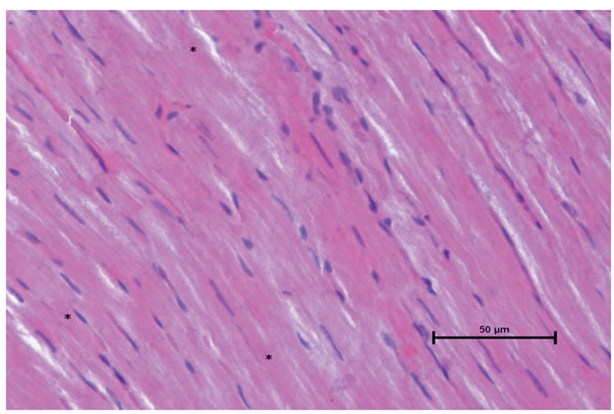

Figure 3. Photo micrograph of Control group. Note: Myofibrils (star) are seen (Hematoxylin-eosin staining, original magnification X 40).

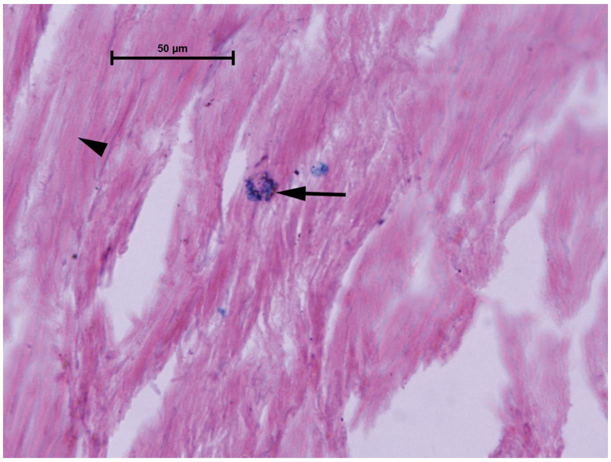

Figure 4. Photo micrograph of Experimental (Exposed to magnetic field) group. Note: Myofibrils (arrowhead) and iron accumulation (arrow) are seen (Perls' Prussian blue staining, original magnification X 40).

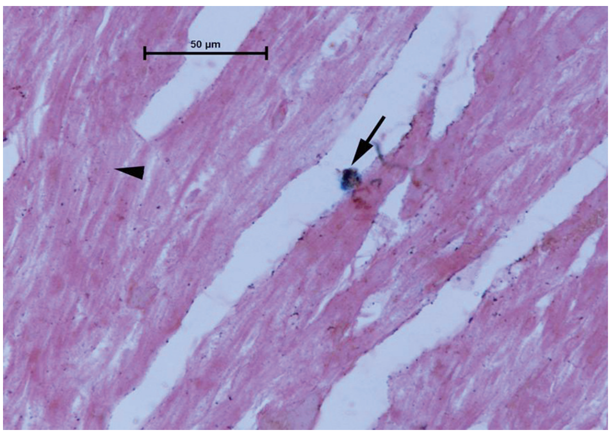

Figure 5. Photo micrograph of Sham group. Note: Myofibrils (arrowhead) and iron accumulation (arrow) are seen (Perls' Prussian blue staining, original magnification X 40).

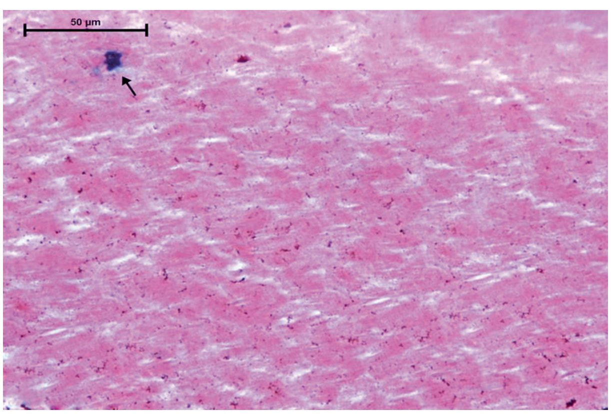

Figure 6. Photo micrograph of Control group. Note: Iron accumulation (arrow) are seen (Perls' Prussian blue staining, original magnification X 40).

DISCUSSION

Low frequency magnetic fields are widely applied in electrical appliances and different equipment such as television sets, computers and kitchen appliances. Recently, low frequency magnetic field has been considered to be a therapeutic agent and it has started to be more and more commonly used in medicine.[14] Electromagnetic fields have adverse effects as a result of widespread use of electromagnetic energy on biological systems. In recent years, a large number of multidisciplinary investigations led to the increasing awareness of the existence of multiple effects of MF in biological systems.[19] The responds of acute cardiovascular system to an electric and magnetic field is still being analyzed.

Several experimental and biological studies have dealt especially with increased incidence of various types of cancer.[1,11] In vivo and in vitro investigations claim that extremely low-frequency magnetic field produced a genotoxic effect, originating from types of free radicals.[12,13] For biological effects of free radicals, especially ROS may produce cellular and toxic effects such as lipid peroxidation in cell membrane, protein degradation, enzyme inactivation.[12,13] Iron is an essential element in a variety of vital processes, including respiratory electron transfer, oxygen transport.[5] However, when iron reacts with H_2O_2, hydroxyl radicals are produced via a Fenton-type reaction. So, iron can catalyze the production of free radicals. Iron ions are strong catalysts for the peroxidation of membrane lipids, and give rise to membrane damage.[7,9,10] Ferritin is the protein responsible for iron storage.[6] Cellular uptake of circulating excess iron results in increased formation of ferritin and hemosiderin found in highest concentrations in parenchymal tissue of several organs. Increased iron content of cells and tissue may increase the risk of cancer. In particular, high available iron status may increase the risk of a radiation-induced cancer. Biological studies have not yet provided direct evidence for a link between electromagnetic fields and cancer.[19] Shao T said the reasonable to link the biological effects of electromagnetic fields with ferritin gene expression and ferritin synthesis which are mainly regulated by iron, hormones and cAMP and eventually the EMF-cancer link.[20]

Lai et al., reported that free radicals and changes in oxidative state in cells could play an important mediating role in some biological effects of nonionizing electromagnetic fields.[12] Certainly, cellular iron metabolism affects these processes. Genetic damage in cells can lead to malignancy and cancer. However, excessive cumulative genetic damages could also result in cell death.[12] An epidemiologic study by Savitz et al. on cardiovascular disease mortality in a cohort of electric utility workers showed an association between exposure to extremely low frequency (ELF) magnetic fields and cardiovascular disease mortality.[18]

In conclusion, extremely low frequency magnetic fields are used in daily life. The effects of ELF-MF have been investigated on heart muscle tissue limited number of histological studies. In our study was to investigate the possible effects of EMF on heart, and cardiac muscle tissue of the intended relationship with iron status. As a result of our study, in the cardiac tissue of rat exposed to extremely low frequency magnetic fields, there was no change detected in the light microscopic study. In this investigation we demonstrated that no differences between control, sham and experimental group rats have been observed in the iron stain of heart tissues. Administered dose and duration for the magnetic fields effects on the heart, although important, further studies are needed to demonstrate whether the ELF-MF exposure can induce adverse effects on the cardiac tissue.

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