

Chronic low-frequency electromagnetic field exposure before and after neonatal life induces changes on blood oxidative parameters of rat offspring

 Zozan Guleken

Department of Physiology, Faculty of Medicine, Uskudar University, Istanbul, Turkey

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Abstract

Aim: It is aimed to investigate the chronic effects of extremely low-frequency magnetic fields (ELF-MF) exposure on blood oxidative and anti-oxidative levels. Rat offsprings during intrauterine (IU) and extrauterine (EU) period exposure to chronic 50 Hz electromagnetic fields included to the study to see the ELF-MF induced changes in blood biochemical levels. To evaluate the cell's physiological response, the levels of glutathione (GSH) and malondialdehyde (MDA) were measured as a label. Recently, the use of electromagnetic (EM) energy has increased in worldwide. Numerous reports indicate that prenatal and postnatal period repetitive ELF-MF leads to health-related issues on physiological responses and affect the activity of the enzymes.

Materials and Methods: Pregnant female Wistar Albino rats (n = 21), weighing 250 – 300 g were randomly divided into MF exposed group (Group I) and sham group (Group II). Eleven pregnant females were exposed to MF for 24 hours a day for IU exposure and exposed to 50 Hz 500 μ T ELF-MF radiating magnetic field cages for 24 hours for the duration of pregnancy for 21 days. Following the birth, first generation newborn rats (n=11) were continued to expose 50 Hz 500 μ T ELF-MF, 24 hours, for additional 50 days in EU period. Sham group (n=10) without any radiation. Groups' blood samples were collected and analyzed. The levels of an oxidative stress marker MDA and antioxidant scavenger GSH activities were investigated.

Results: Long-term exposure to ELF-MF increased MDA concentration in blood plasma of rat offspring and decreased GSH levels significantly. No correlation was shown between MDA and GSH levels.

Conclusion: Long-term ELF-MF exposure causes oxidative stress that results an increase on lipid peroxidation and a reduction on antioxidative parameters of blood tissue.

Keywords: Blood; ELF-MF; GSH; magnetic field; MDA; postnatal exposure; prenatal exposure

INTRODUCTION

Until fifty years ago, the natural level of the electric and magnetic fields in the atmosphere was quite low. The use of electromagnetic energy that generates ELF-MF has become widespread with technological applications and industrialization, and an environmental increase has occurred in every frequency of EM fields affecting all living organisms' biological systems. Considering that, the use of EM areas will increase even more in the future, the importance of the subject increases. However, all of the living organisms have to be constantly close to the magnetic field exposure with a high risk (1). ELF-MF is expressed as 300 Hz up radiation, which may interact with specific biomolecules. Magnetic field induces electrical current in the body. Additionally, studies had shown that electromagnetic field exposure has harmful biological impacts on human health. (2).

Studies showed that ELF-MF affects intercellular activity of membrane cell, hormone secretion, and immune system, and it leads to effects on skin, headache, cardiovascular problems, gastrointestinal diseases, psychiatric problems, and anomalies on the development of embryos (3). In addition, studies showed that ELF-MF exposure has effects on normal cell proliferation and disrupts their normal development (4). Researchers reported that the association of children with exposure to a magnetic field greater than 0.4 mT increased the risk of leukemia which is a major childhood disease. (5). Similarly, clinical reports with findings from ELF-MF showed a significant increase in deaths resulting to that leukemia and lymphoma cancer (6). Some studies have used animal embryos to compare the developmental changes between ELF-FM exposure and unexposed healthy controls with the results in increased abnormalities in animals' embryogenesis (7,8).

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Corresponding Author: Zozan Guleken, Department of Physiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey

E-mail: zozanguleken@gmail.com

Exposing to electrical field has significant impacts on biological materials via changing electrical signals. Low frequency and low intensity EM fields influence the movements of molecules and ions resulting changing the uptake and secretion of ions which lead to improve the balance of ions in the body. Membrane interaction with ELF-MF causes a change on the cell electrical signal order. Electrical signals have important role for the transmission of information in the process of biological events. This interaction is sufficient for the critical biochemical changes of blood (9). Clinical evaluation of ELF-MF exposure resulted in the numerous changes of white blood cells and red blood cells (10). Recent studies had shown that exposure to an ELF-MF less than 200-300 Hz may alter changes on cell metabolism resulting in to generation of free oxygen radicals causing oxidative stress (11). Free radical and metabolite imbalance causes oxidative stress. These products named as oxidants or reactive oxygen species (ROS) and the protective elimination mechanism towards these products referred as antioxidants. The homeostasis of oxidants and antioxidants is important for the cells, biomolecules and has potential impact on membrane macromolecules such as lipids, proteins and DNA (12). Oxidative attack causes on the main scaffold of membrane such as proteins and lipids, may lead to modification of these molecules, which may increase the risk of mutagenesis (13). Malondialdehyde (MDA) level is an important biomarker for the determination of destruction on lipids of organism (14). MDA levels were analyzed to identify oxidative lipid peroxidation and DNA damage on blood tissue. To protect the body against the potential of ROS, cells possess several antioxidant enzymes such as glutathione peroxidase (GPx), which reduces H_2O_2 to H_2O using GSH as an electron source to eliminate the stress, produced by oxidative stress (15). For this reason, we demonstrate the level of GSH in erythrocytes.

However, long term and chronic effect of MFs are inconclusive and not significant. However, there is not enough study about IU and EU exposure to low frequency of MF and bimolecular interaction (16). As it is known, IU life indicates mitosis and meiosis that play role in cellular process. This process of evolution appears to be affected external stressors such as physical agents, heat, or chemical agents and as well as MF exposure (17). Animal studies had shown that exposure to ELF-MF had significant risk impaired for fetal development. (18). The studies in this field clarified an increase on the imbalance of cellular progress such as apoptosis of cell proliferation. However, long-term effects of human-made EMF on human health are not established. For this reason, we prepared an experimental design with an externally applied electric field to study with the IU and EU stage of rat pups to see the impact of ELF-MF for a long time on whole body with blood oxidative parameter. However, there is not enough study about the long term and chronic impact of ELF –MF exposure on the physiology of blood tissue.

Statistics

We used t-test (IBM SPSS Statistics 25) to compare the groups. All results are expressed as a mean \pm deviation (SD). Differences with a $p < 0.005$ were regarded as significant.

MATERIALS and METHODS

Animals

Sprague Dawley female rats (250–200 g), obtained from Bezmialem University Experimental Animals Research and Implementation Centre. Experimental protocols were approved by Bezmialem University local ethics committee (2020/95). The animals were fed with standard rat chow and able to access water ad libitum. They were housed in the standard cages one by one, under controlled environmental conditions with an ambient temperature of $(22 \pm 2^\circ C)$ a 12-h light/dark cycles and 60 % humidity, during the experiment (lights on from 6:00 AM to 6:00 PM). Accordance to the Declaration of Helsinki and with the guidelines of the care and use of laboratory animals.

Experimental design

Pregnant female rats weighing ($n = 2$) 250 – 300 g were randomly distributed into two main groups: Sham group ($n = 1$) and magnetic field (MF) induced ($n = 1$) group. Pregnancy of rats was confirmed with smear. MF exposed group (Group I) was placed into 50 Hz 500 μ T ELF-MF radiating magnetic field cages for 24 hours for the duration of pregnancy (21 days) for IU period. Following the birth, newborn rats Group I ($n=11$) were exposed to 50 Hz 500 μ T ELF-MF, 24 hours a day, for additional 60 days for EU period. Group II ($n=10$), was not exposed to the ELF-MF, and subjected to same growth conditions with the study group. After the 60th days, all animals were sacrificed with intramuscular ketamine (35 mg/kg), and xylazine (5–10 mg/kg,) injection which purchased from Ted Ecza Deposu and body trunk blood was obtained by cardiac puncture and collected for the measurement of blood GSH and plasma MDA (19).

ELF-MF Exposure

ELF-MF was produced using Merritt coil system, which produce 50 Hz frequency and 500 μ T field surrounded in a frame. Internal coils consisted of 11 turns, and external coils had 26 turns. Each pregnant rat was placed into the separate cages, and the cages were located on the shelves of the ELF-MF producing system within the study period. During the exposure, the magnitude of the ELF-MF was measured for every six-minutes with a magnetic field probe (Narda EHD-50D, Germany).

Blood collection, preparation of the hemolysate and plasma

Blood was collected via cardiac puncture from anesthetized animals and collected in two separate tubes containing ethylene diamine tetra acetic acid (EDTA). Plasma was separated from red blood cells by centrifugation at 3000 rpm for 15minutes, then immediately aliquoted and stored at $-80^\circ C$ until analysis. Erythrocytes were washed three times with ice-cold phosphate- buffered saline at pH 7.4

and centrifuged to get rid of the ghost and intact cells and stored at -80°C until analysis (20).

MDA assay

Plasma MDA concentration was determined as previously described (21). To measure the MDA level, 0.5 ml of plasma was mixed with 2 ml of thiobarbituric acid (TBA) with a reagent TBA (0.375%), TCA (15%) and hydrochloric acid (HCl) (0.25 N), before mixture was cooled and centrifuged and obtained in boiling water bath for 15 minutes. The supernatants of the mixture were obtained, and then the formed pink colors optical density was read at 535 nm. The absorbance against the standard graph was used to determine the level of MDA in samples. The optical density was expressed as the level of serum MDA in the given sample. Lipid peroxide levels were expressed as micromol/L (22).

GSH assay

Erythrocyte GSH levels were determined as previously described (22). 5,50-dithiobis-(2- nitro benzoic acid) (DTNB) is added to sulfhydryl compounds with the development of stable yellow color. Metaphosphoric acid was used to precipitate proteins in the homogenate then removed after centrifugation. 100 ml of the supernatant, 120 ml of $\text{Na}_2\text{H}_2\text{PO}_4$ and 8 ml of DTNB solution were subsequently mixed and after 4 hour of incubation period at 412 nm absorbance values were measured. The unit of the results was stated as $\mu\text{mol} / \text{gHb}$. The concentrations of MDA and GSH in the blood of study groups are illustrated in Table 1.

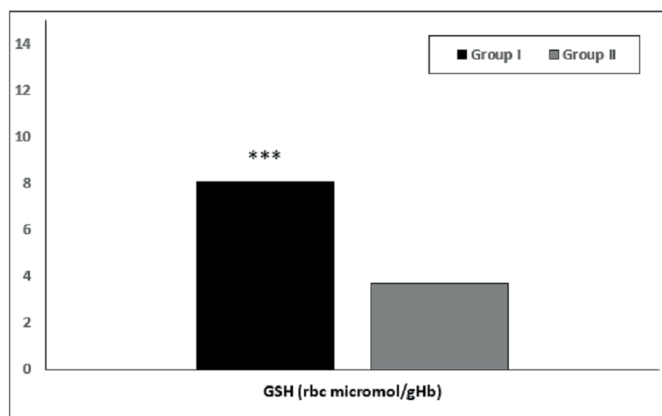
Table 1. The concentrations of MDA and GSH in the blood of study groups			
	Group I (-)	Group II (+)	p value
MDA (plasma micromol/L)	5.51 ± 103	6.35 ± 0.93	$p < 0.01$
GSH (rbc micromol/gHb)	5.82 ± 1.43	6.20 ± 1.27	$p < 0.001$

Each group consists of 11 rats. Group I was exposed to 50 Hz 500 μT ELF-MF radiating magnetic field cages for 24 hours for the duration of pregnancy (21 days) for intrauterine (IU) period and following the birth, for additional 60 days for extra uterine (EU) period. Group II was not exposed to the ELF-MF, and subjected to same growth conditions with the study group for the same period

RESULTS

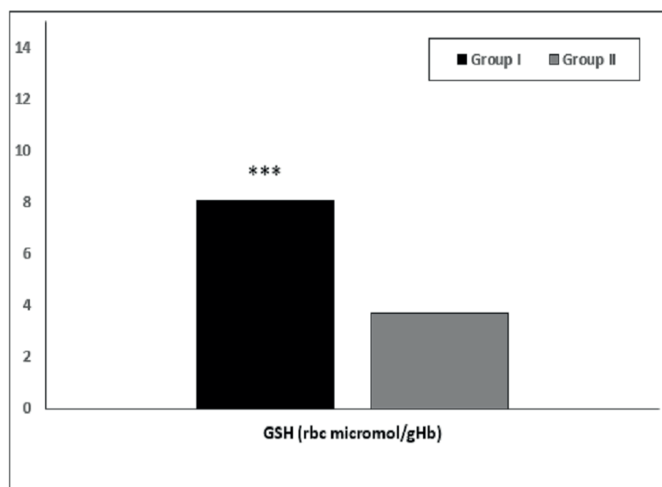
In this study ELF- MF radiating protocol was composed by magnetic field cages. One of grouped pregnant animals was exposed to 50 Hz 500 μT ELF-MF for 21 days until birth and rat offsprings exposed to MF additional for 50 days after birth. However, other pregnant group and their offsprings did not exposure to MF until the same experimental period before birth 21 days and after birth for 50 days. The long-term effect of ELF-MF on blood oxidative parameters before and after birth on puppies

has been investigated. We measured blood MDA level and serum GSH levels of puppies that exposed to ELF-MF in IU and EU period to observe oxidative effects. From Figure 1 it can be seen that compared to the Group I (Sham exposure group). (5.51 ± 103) with mean \pm SD the plasma MDA level of Group II (6.35 ± 0.93) was significantly increased ($p < 0.01$). Figure 2 illustrates the comparison of blood GSH levels of Group I and Group II. When we compared groups, blood GSH levels (5.82 ± 1.43) of Group I and ELF-MF exposed Group II (6.20 ± 1.27), there was a decreased GSH level. These results were statistically significant ($p < 0.001$).



Results were expressed as mean \pm SD. The measurement of MDA 18.01 ± 2.97 for ELF-MF exposure group (Group I) and 10.75 ± 2.28 for sham exposure group (Group II). The statistical significance was signified as less than or equal to $p < 0.01$ **

Figure 1. Malondialdehyde (MDA) levels in blood plasma



Results were expressed as mean \pm SD. 22.78 ± 6.37 for ELF-MF exposure group (Group I), 16.44 ± 1.38 for sham exposure group (Group II). The statistical significance was signified as less than or equal to $p < 0.001$ ** . Each group consists of 11 animals

Figure 2. Glutathione GSH) levels in erythrocytes

DISCUSSION

It was hypothesized that prolonging ELF-MF exposure in IU and EU period would have affects on infant's cell structure

and biochemical blood parameters hence directly on oxidant and antioxidant parameters. For this purpose, to measure, the effect of MF on blood parameters we assessed by determining the blood MDA level and serum GSH levels of rats' pups exposed to ELF during and after the prenatal period.

ELF-MF originates from residential and industrial power lines, medical devices and appliances of household. It has been reported that it is produced by a large variety of electronic devices in everyday use. This repeatedly exposure results in a variety of biological effects that may prompt to a number of changes in biochemical blood levels which may lead to metabolic and biochemical processes (23). In case of continuous exposure to or induction of electric fields, various biological processes can be initiated. It is emphasized that chronic exposure may lead to pathophysiological changes in the function of organs such as ren, liver and testis (24-26).

Studies showed that oxidative stress leads to various diseases resulting in, aging, pulmonary fibrosis, atherosclerosis, cancer, and neurodegenerative diseases (26,27). Briefly, the interaction between the mechanism of pathobiology and oxidative stress has not known yet.

In this study, GSH activity was measured using enzymatic reaction with glutathione reductase ($\mu\text{mol NADPH}_2/\text{mL medium}$). GPx, reduces H_2O_2 to H_2O via GSH (28). GSH is an antioxidant that may deactivate reactive oxygen species. As it is known that GSH levels are indicative of oxidative load. In this study the duration of ELF- MF might increase oxidative load. Similarly, previous animal studies have been noted that GSH levels were decreased in response to ELF-MF exposure either in a short time or long-time manner in different tissues and regions. (29,30). Also, there are some cell culture studies that provides increased levels of ROS as a result of ELF-MF exposure (10). The wide spectrum of the frequency and amplitude modulation of ELF-MF used in our experiment could create the possibility for resonant interactions of ions present in the studied molecules. Thus, chronic ELF-MF exposure results in decreased antioxidant enzymes.

Decreased MDA levels prevents lipid membrane peroxidation by its direct scavenging free radical actions. In this study, MDA levels of ELF- MF exposure group were significantly increased compared to the sham exposure group. Obtained results can be discussed because of the resonant interactions of ELF-MF with biological systems (31). It is known that ROS are continuously produced during aerobic metabolism (32). A number of enzymes and vitamins counteract oxidative damage caused by free radicals. This interaction causes an imbalance between oxidative and antioxidative process. The activation in the peroxidation of free radicals cause peroxidation of lipids on membrane structure. This leads to serious membrane, cell and organelle damages by stimulating autocatalytic chain reaction (33). However, previous studies had

shown that newborn rats exposed to ELF-MF might have genotoxic and cytotoxic damages in bone marrow and brain cells (34). Our results are consistent with correlative with these studies. Controversially, it is claimed that ROS are not only injurious by-products of cellular metabolism but also essential for cell signaling and regulation (35). Additionally, animal experiments have been shown that 50 Hz ELF-MF exposure elicits redox and trophic response in rat-cortical neurons (36).

IU and EU period of rat pups have been chosen for this study since; prenatal and natal periods have a high capacity to undergo re-organization proliferation and differentiation. This study provides convincing evidence that long-term ELF- MF exposure of rat pups before and after gestational period leads to appreciable long- term deficit in blood parameters.

CONCLUSION

It was aimed to research the cellular response to exposed MF in the most critical stages of the development processes, during IU period and EU period in early childhood in an animal experimental design. Herein, this research provides whether chronic EMF exposure at 50 Hz may result in an imbalance between oxidative and antioxidative blood and plasma parameters of rats offsprings. Additionally, present data strongly suggest that ELF-MF has an important role in regulation of enzyme activity and has effects on biochemical processes, possibly by improved production of ROS. Acute and chronic exposure cause structural deviations in biological systems that result in disruption on the general physiology and cellular homeostasis of the organism. Additionally, to understand oxidative load under chronic ELF-EMF exposure before and after IU period further researches are needed.

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Ethical approval: Accordance to the Declaration of Helsinki and with the guidelines of the care and use of laboratory animals our study was accepted by properly appointed and approved by the Bezmialem University Animal Care and Use Committee. (2020/95).

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