ORIGINAL ARTICLE

Evaluation of Liver Volume and Its Megakaryocyte's Population in NMRI Mice Fetus Exposed to Electromagnetic Field

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Abstract

Background: During the recent decades, a lot of studies were conducted to asses the effect of electromagnetic fields on fetal developmental disorders, sterility, and a variety of neoplasms. The aim of the present study was to determine the effect of low electromagnetic field (LEMF) on the liver's volume and the number of its megakaryocytes in NMRI mouse fetus.

Methods: LEMF of 50 HZ frequency was used and 66 three month old NMRI mouse embryos were divided into 6 groups. The experimental groups were exposed to LEMF and then an embryo was randomly selected from each mother and its liver was extracted and fixed in formalin. Cell counting and volume evaluating were done by stereological methods and the data were analyzed.

Results: Although the number of liver megakaryocytes and the embryos weight in all experimental groups as compared to sham and control groups reduced but the differences were not significant.

Conclusion: The results of this study revealed that in the pregnant mice exposed to LEMF, irrespective of the length of pregnancy, there was not a significant change in the liver volume and the number of liver's megakaryocytes in NMRI mouse fetus.

Keywords: Electromagnetic field; Megakaryocyte; Liver; NMRI Mouse; Embryo

Introduction

During recent decades, ever-increasing usage of electromagnetic field producer instruments has attracted the researcher's attention to probable risks of these instruments for human health.[1,2] In normal life environments, the commonest frequency used by most electrical instruments is about 50-60 Hz and 6-10 amperes current; thus, the electromagnetic field intensity around these instruments depends on the distance and current ranging from 0.1-8 militesla.[3] In this relation, in classification of these waves like LEMF wave, they belong to high wave length domain such as infra-red, microwave and radio. Also, in classification of these waves as temperature producer waves, they belong to intermediate and low levels.[4] The results of recent studies about the side effects of electromagnetic fields indicate that even a little change in the electromagnetic field intensity as small as a few militesla will produce different biological effects.[5-7] It was shown that extra low frequency (50-80 Hz) electromagnetic fields are the most dangerous ones and great biological destructive effects may be provided in these frequencies. For example, electromagnetic fields in 50-60 Hz and 23 miliampere current will cause painful shock and severe cardio-respiratory problems.[5] LEMF (27-250 MHz) has a curative effect because it has a power to make tissues warm[6] and in total there are several reports that indicate a linkage between exposure to power frequency LEMF (50-60 Hz) and abnormalities in the early embryonic devel-

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opment, sterility, different neoplasms specially hematopoitic and lymphatic tissue, and nervous, sleep, gastrointestinal and cardiovascular disorders.[7-10] Although recent studies showed that LEMF has some effects on the rate of brain growth and liver neoplasms,[11] leukemia and lymphoma in military men exposed to permanent LEMF have been reported several times.[12] In-vitro studies on LEMF doubtlessly have shown increasing cell ($Ca2^{+}$),[13,14] free radicals establishment, and activating jumping genes.[15,16] According to Savitz and Poole's case-control long-term study on children suffering from cancer, in children exposed to LEMF, the chance of affliction with cancer has been more than that in others. They reported more cases of lymphatic tissue neoplasms.[17,18] Lino et al. demonstrated that it increases ESR[19] and concurrently decreases hematocrit. Considering the fact that LEMF aims at lymphatic and hematopoietic organs and the researchers' lack of concern about the effect of LEMF on the liver volume and number of its megakaryocytes, in this study we have evaluated the effect of 50 Hz LEMF in 5 Militesla on liver's embryonic development as an hematopoietic organ, considering its volume and the number of its megakaryocytes. The 5 Militesla LEMF is commonly produced by most home or work appliances.[4]

Materials and Methods

This study was carried out on 66 female 9-12 weeks old NMRI mice, provided from Pasteur Institute, Tehran, Iran. The mice were individually housed in polypropylene cages (30× 20× 15 cm) with drinking water and food ad libitum. The animals' rooms were artificially illuminated under 12-h light/dark cycle (light during 7.00 A.M. to 7.00 P.M.), with a temperature of 20-22°C and humidity of 45-50%. After a week, the mice were fertilized and randomly divided into 6 groups (Table 1). In this experiment, each time two mice were exposed to 5 Militesla (50 Hz) LEMF for 6 hours in each day, for 3 weeks. Beginning of the pregnancy was determined by daily evaluation of sperm presence in vaginal smear by light microscopy analysis (ZEISS, ×100). One hundred and eighty eight offsprings of this experiment were weighted by Triple Beam Balance Lab Scale with sensitivity of 0.01 gr and from each mother, 1 embryo was randomly selected. The animal was sacrified and its liver was extracted and kept in 4% formalin. The liver sections were prepared according to stereological methods and stained with H&E.

The LEMF exposure conditions were as follows:

1- Power supply; 220 Volts in; and 25 Volts out; and permanent current intensity of 3 Amperes. 2- Multimetere instrument to control the permanent current intensity entering the instrument. 3- A 50 Hz sinusoidally oscillating LEMF was produced by a 380 round coil twisted around a cylinder (19 cm diameter and 15.5 cm length) containing a chamber to keep the mice in exposure. 4- A Teslametere-51662, (compensation, UK) to precisely evaluate the magnetic field intensity in the chamber where animals were exposed. 5- A chamber to keep the animals exposed to LEMF in the center of the cylinder, producing the maximum (5 mT) even LEMF.

The intensity of LEMF in the chamber was evaluated and confirmed by Teslametere. All the instruments were set up and tested by physicists of Arak University and Arak University of Medical Sciences, Physics and Medical Physics Department. During the experiment, the current intensity was ±0.01 Ampere and the maximum changes in the magnetic field was ±0.1 mT, which could be overlooked.

To determine the stereological analysis of megakaryocytes population, we used Cavaliery method to numerically evaluate the offspring's liver megakaryocytes.[20] Stereology is originally defined as the spatial interpretation of sections and materials. Cavaliery method was used to provide the same chance for random selection of the samples, sections, microscopic fields and conformity with Grid points.

Table 1: Specifications of different groups participated in this study

Groups	Specifications	No
Sham	Located in off system	1
Control	Without any intervention	2
Experiment 1	Exposed to LEMF in (7-11 Days) liver and yolk sac formation time	3
Experiment 2	Exposed to LEMF in (10-14 Days) liver formation time	4
Experiment 3	Exposed to LEMF in (13-17 Days) liver and bone marrow formation time	5
Experiment 4	Exposed to LEMF in (17-21 Days) hematopoiesis starting time	6

As the mean length of each liver in newborns was 7.5 mm=7500 µm and the diameter of smallest megakaryocytes measured by Graticule was about 30 µm, 750 sections (10 µm thickness) were provided. In this method, 15 double serial sections (leaving 50 sections) were separated from each liver and stained.

To count the number of megakaryocytes, physical dissectors[21] were used and concurrently two random images belonging to two sequential sections were projected over the stage, the first as the reference and the next as a look up. To gain the proper estimation of the cells, 5 microscopic fields were chosen, projected and counted from each section. A transparent image containing 13×13 mm frames (Figure 1) was conformed randomly over the microscopic fields projected images (Figure 2) and then the cells were counted according to stereological principles and comparative number evaluation of each liver in the same distinct volume[22] was assessed, using a formula (No. 1).

Formula No. 1: To account the number of Megakaryocytes in volume unit.[23]

$$Nv = \frac{1}{\frac{a}{f}} \times \frac{\sum Q -}{\sum P}$$

$$M^2$$

Nv, number of Megakaryocytes in volume unit, ΣQ, No. of All megakaryocytes accounted in all sections, ΣP, No. of All frames containing megakaryocytes accounted in all sections. H, Dissector height, a/f, Each frame area by Micrometer, M, linear magnification of each image.

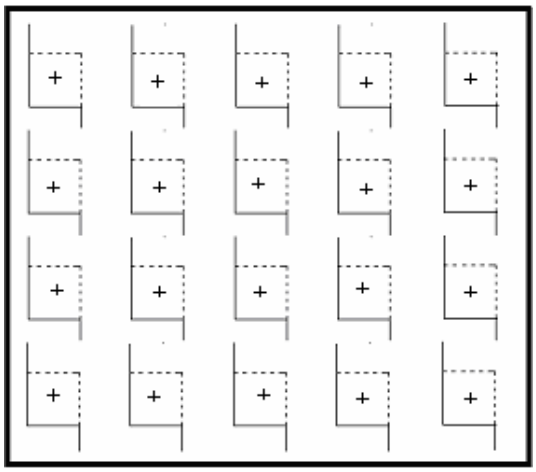

Figure 1: A transparent containing 20 frames (13×13 mm) to conform to liver microscopic images to account the number of megakaryocytes.

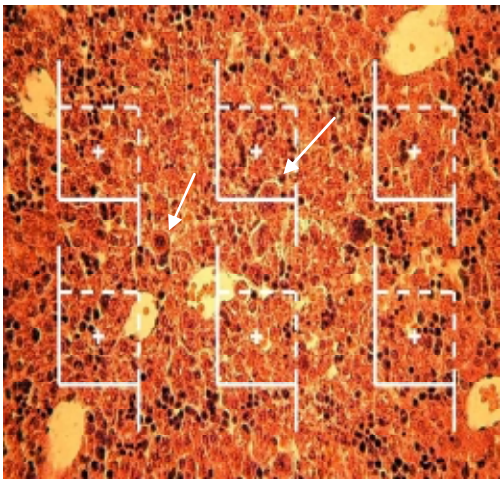

Figure 2: The transparent image conformed to liver microscopic Image containing several megakaryocyte (Flashes).

Stereological coefficient of error is a useful formula that helps to understand the validity of stereological estimation that was applied. The maximum coefficient of error was lesser than 10%. This coefficient can be provided by formula (No. 2).[23]

Formula No. 2: Formula for accounting the Coefficient of error of megakaryocytes.[22]

$$estCE_{NV}=\left[\frac{K}{K-1}\left(\frac{\sum_{I=1}^{K}LiverU^{2}}{\sum_{I=1}^{K}LiverU\sum_{I=1}^{K}liverU}+\frac{\sum_{I=1}^{K}MV^{2}}{\sum_{I=1}^{K}MV\sum_{I=1}^{K}MV}-2\frac{\sum_{I=1}^{K}liverUxMV}{\sum_{I=1}^{K}liverU\sum_{I=1}^{K}MV}\right)\right]^{\frac{1}{2}}$$

$estCE_{NV,}$ Coefficient of error of approximate; number estimation of megakaryocytes in volume unit, MV, The number of all megakaryocytes counted in all frames; Liver U, The number of frames containing any number of megakaryocytes, K, The number of all sections being accounted, Nv, The number of megakaryocytes in Unit volume.

SPSS software (version 14, Chicago, IL, USA) was used for statistical analysis. According to stereologic evaluation of the mean volume of 66 livers in the newborns in the different groups, the data were assessed by Kuruskal-wallis non- parametric test.

Results

Although the mean number of megakaryocytes in all the experimental groups comparing to sham and control was decreased (Table 2), this reduction was not significant (p=0.10). The least number of liver megakaryocytes belonged to 10-14 days group and the most to 13-17 days group (Table 3). Comparison all the experimental groups with sham and control ones, we found that the liver volume in the experimental groups reduced and the least volume belonged to 10-14 days group; this difference was not statistically significant (p>0.05).

Discussion

This experiment revealed that the mean number of megakaryocytes and liver's volume in all experimental groups decreased but the reduction was not significant. It seems that disregarding the pregnancy age, LEMF had the same effects on all embryos. Recent studies in cell death induced by LEMF in different types of cells showed that LEMF can play a part in producing a kind of cell death inducing signals;[23,24]

Table 2: Liver Megakaryocytes mean estimation in all groups per unit volume

Groups	Mean (μm^3)	Coefficient of error	Mode (μm^3)	Min (μm^3)	Max (μm^3)
Sham	45.09×10^{-6}	0.122	65.51×10^{-6}	18.77×10^{-6}	75.09×10^{-6}
Control	48.75×10^{-6}	0.214	36.27×10^{-6}	21.90×10^{-6}	59.45×10^{-6}
7-11Days	36.45×10^{-6}	0.112	36.24×10^{-6}	31.29×10^{-6}	47.80×10^{-6}
10-14 Days	23.36×10^{-6}	0.165	25.97×10^{-6}	98.41×10^{-6}	38.51×10^{-6}
13-17 Days	33.88×10^{-6}	0.111	39.54×10^{-6}	16.87×10^{-6}	53.06×10^{-6}
17-21 Days	25.23×10^{-6}	0.113	26.94×10^{-6}	11.45×10^{-6}	39.77×10^{-6}

Although the mean number of megakaryocytes in all experimental groups comparing to sham and Control is decreased (Table 2) But this reduction is not significant (P=0.10)

Table 3: Stereologic Mean estimation of embryo's liver volume

Groups	Mean Volume (μm^3)	Mode (μm^3)	Coefficient of error	MinVolume (μm^3)	MaxVolume (μm^3)
Sham	692×10^{3}	722×10^{3}	0.298	496×10^{3}	103×10^{4}
Control	968×10^{3}	825×10^{3}	0.367	514×10^{3}	112×10^{4}
7-11Days	516×10^{3}	543×10^{3}	0.139	531×10^{3}	865×10^{3}
10-14 Days	535×10^{3}	565×10^{3}	0.139	489×10^{3}	715×10^{3}
13-17 Days	628×10^{3}	611×10^{3}	0.234	328×10^{3}	686×10^{3}
17-21 Days	487×10^{3}	498×10^{3}	0.281	327×10^{3}	973×10^{3}

Comparing all experimental groups with sham and control, the liver volume in experimental groups is reduced and the least volume belongs to (10-14 days) group, this difference is not significant (p>0.05).

these signals would affect the cell cycle finally. The products of these inducing signals were known as stress proteins.[25] Today, it has been argued that there is a variety of these proteins in different cells and they are not only able to cease the cell cycle but also they increase the velocity of cell cycle. Although LEMF in some kind of cells can take part in micronuclei production and stop the cell cycle that will lead to cell death,[23,24] recent studies have shown that in neurons it may induce production of a kind of opioid and in mast cells it may induce cell division signals.[26] Megakaryocytes have two life stages. In the first stage following sequential mitotic divisions in their progenitors, a giant mature megakaryocyte will appear and in the second stage mature megakaryocytes break into pieces and loosen their nucleus, the particles being called platelet. Cell division in megakaryocyte's progenitor cells and appearance of the platelets are under the effect of prothrombin production in both progenitor and mature megakaryocytes. The extension of prothrombin time (a decrease of INR), decrease in the activity of factor Xa and decrease in the platelets level were observed in the animals after their exposure to low magnetic field.[27] According to another study, megakaryocytes exposed to LEMF will produce a kind of inducing signals[28] that reduce the production of prothrombin, interleukin III and stem cell factor,[29] among which prothrombin reduction has the most adverse effects on megakaryocytes. Of course, we should consider that exposure to electromagnetic fields in different situations may lead to different results. For example; exposure to homogeneous magnetic fields as low as 0.005 T leads to decreased platelet count; increased platelet aggregation; but increased prothrombin and partial thromboplastin times; decreased fibrinogen, and increased fibrinolysis.[30]

As prothrombin affects both life stages of megakaryocytes, leading to the reduction of megakaryocytes, it seems that in this experiment 5 Militesla LEMF did not affect the production of prothrombin, interleukin III and stem cell factor, because changes in the number of megakaryocytes was not significant. However, the non-significant decrease in the number of megakaryocytes in 10-14 days group might be due to apoptosis[31] (Table 2). The results of this study revealed that in the pregnant mice exposed to LEMF, irrespective of the length of pregnancy, there was not a significant change in the liver volume and the number of liver's megakaryocytes in NMRI mouse fetus.

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