

## Research Article

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# Effect of 50-Hz Magnetic Fields on Argyrophilic Nucleolar Organizer Regions in Bone Marrow Cells

Mohammad Rafiee<sup>1</sup>, Mohammad Abbasi<sup>2</sup>, Mohammad Yousef Alikhani<sup>3</sup>, Safoora Nikzad<sup>4</sup>, Alireza Zamani<sup>5\*</sup>

<sup>1</sup>Department of Medical Laboratory Sciences, School of Paramedicine, Hamadan University of Medical Sciences, Hamadan, Iran

<sup>2</sup>Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

<sup>3</sup>Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

<sup>4</sup>Department of Medical Physics, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

<sup>5</sup>Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

**\*Corresponding author:**

Alireza Zamani,  
Department of Immunology,  
School of Medicine,  
Hamadan University of  
Medical Sciences, Hamadan,  
Iran  
Tel: +98 813-8276295-8  
ext. 442,  
Fax: +98 813-8276299,  
Email: a\_zamani@umsha.ac.ir

**Abstract**

**Background:** Exposure to an increasing amount of extremely low-frequency magnetic fields (MFs) causes some adverse effects. Considering a direct association between the sizes and numbers of argyrophilic nucleolar organizer regions (AgNORs) and cell proliferation, this study was conducted to evaluate the effect of 50-Hz MFs on the AgNORs of bone marrow (BM) cells.

**Methods:** Eighty male rats were randomly divided into four exposed groups and one control group. The exposed groups received 50-Hz MFs with magnetic flux densities of 1, 100, 500, and 2000  $\mu$ T two hours a day for two months. BM cells were aspirated from sacrificed rats' femoral bones, smeared on glass slides, and then stained with silver nitrate for AgNORs. The area (AA), length (AL), and number (AN) of AgNORs were calculated by a microscope equipped with a camera and Scion Image software in 100 BM cells of each rat. The mean of AA, AL, and AN was computed for each group.

**Results:** AA, AL, and AN significantly reduced in the 1  $\mu$ T group compared with 2000  $\mu$ T and control groups. Eventually, there was a nonlinear relationship between the effect of 50-Hz MFs and magnetic flux densities.

**Conclusion:** Overall, 50-Hz MFs with a magnetic flux density of 1  $\mu$ T reduced AgNORs in BM cells. However, 100, 500, and 2000  $\mu$ T did not affect AgNORs. Therefore, 50-Hz MFs with low density may suppress BM cell proliferation.

**Keywords:** Bone marrow, Argyrophilic nucleolar organizer regions, Magnetic fields

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**Introduction**

Nowadays, electrical energy is extensively consumed due to advances in technology, producing a large amount of extremely low-frequency magnetic fields (ELF-MFs) in our environment. Therefore, ordinary electrical appliances (e.g., televisions, computers, refrigerators, ovens, and electric blankets) and high-voltage cables in addition to the natural sources produce ELF-MFs. Furthermore, the health effects and biomagnetic interactions have received attention over the last years, and various kinds of results have been mentioned in this regard. Some studies have reported that ELF-MFs can also affect cell proliferation and apoptosis (1-5).

Argyrophilic nucleolar organizer regions (AgNORs) are DNA sequences of chromosomes 13-15, 21, and 22 of cells and are involved in ribosomal RNA synthesis. In addition, AgNORs are visible as black and dark brown spots in interphase nuclei by silver staining due to their argyrophilic nature (6,7). Previous research showed that

there is a direct relationship between the cellular level of ribosomal RNA and the ability of cells to proliferate, implying that the proliferation capability of cells increases with an increase in the number and size of AgNORs (8). Moreover, some studies reported that AgNOR proteins might have a protective role (9,10).

Bone marrow (BM) is one of the most crucial organs that is characterized by high cell proliferation and is vulnerable to ELF-MFs. Although some studies have focused on the effect of ELF-MFs on the AgNORs and proliferation of BM cells, their results are still controversial (3,4,7,11). An *in vivo* study by Okudan et al demonstrated the effect of 50-Hz MFs with different magnetic flux densities (i.e., 1, 2, 3, 4, and 5  $\mu$ T) on the nucleus size and AgNORs of peripheral blood lymphocytes in mice. They found a nonlinear decline in the nucleus and the AgNOR area (7). In addition, Erdal et al studied the mitotic index (MI) in the BM cells of rats and reported different results. They exposed rats to 50-Hz MF with 1000  $\mu$ T magnetic flux



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density for one day (4 hours, as an acute-exposed group) and 45 days (4 h/day, as a long-term-exposed group). Their results revealed that MI in BM cells significantly decreased in acute- and long-term-exposed groups compared with controls. However, there was no significant change between the above-mentioned groups (11). Another *in vitro* study addressed various magnetic flux densities of ELF-MFs on the proliferation of human breast cancer cells (BT-20), and the results indicated a nonlinear decrease in cell proliferation (3). In contrast, a study investigated the effect of continuous whole-body exposure of newborn rats to 50-Hz MF with 500  $\mu$ T magnetic flux density and reported a nearly three-fold increase in the MI of the exposed BM cells (4).

Therefore, this study aimed to evaluate the AgNORs (i.e., area, length, and number) of BM cells following exposure to various flux densities of 50-Hz MFs.

## Materials and Methods

### Animals

Eighty albino Wistar eight-week-old male rats were purchased from the animal facilities of Hamadan University of Medical Sciences, Hamadan, Iran. The rats were randomly separated into four exposed groups and one control group each containing 16 rats. The rats were kept in plastic cages in an air-conditioned lab at a temperature of 22°C and relative humidity of about 60%. After one week of adaptation, the exposed groups were treated in the middle of solenoids producing 1, 100, 500, and 2000  $\mu$ T magnetic flux densities at 50-Hz MFs from 8-10 am for two months. The controls were also placed in a solenoid when it was unplugged. To activate BM cells, all the rats received human serum albumin. The rats were fed with standard food pellets and drank tap water *ad libitum* (12-16). The protocol of the study was compliant with the national guidelines for using animals and was approved by the Ethics Committee of Hamadan University of Medical Sciences, Hamadan, Iran (Code: UMSHA/P/16/35/9/6829).

### 50-Hz MF Exposure Systems

Exposure units (solenoids) were designed using 200 cm length and 20 cm radius of polyvinyl chloride cylinders by an electrical engineer (Microsystem Company Hamadan, Iran). The cylinders were coiled with two millimeters (in diameter) of copper wires. The solenoids could produce magnetic flux densities of 1, 100, 500, and 2000  $\mu$ T by adjusting different electric currents (0.2, 0.7, 2, and 3 A), electrical voltages (2.7, 6, 37, and 88 V), and turns of wires (600, 600, 1200, and 1200), respectively. All systems were connected with the 50-Hz frequency of power supplies. The rats were placed in the middle of the solenoids in their plastic cages. An ELF-MF Survey Meter (HI-3604, Holaday Ind., USA) was used to measure magnetic flux densities. The background magnetic flux densities in the

animal and experimental labs were  $0.07 \pm 0.03$ , and  $0.09 \pm 0.04$   $\mu$ T, respectively (12-16).

### AgNOR Staining

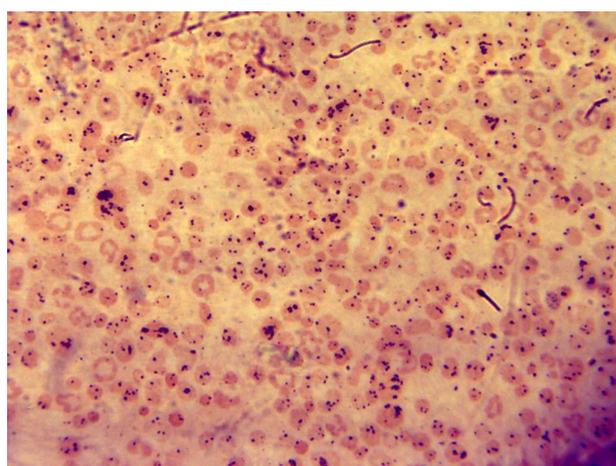
All the rats were weighed and sacrificed, and femoral bones were separated from the rats' legs and washed in normal saline. BM cells were extracted and smeared on glass slides. The smears were then labeled and fixed with methanol. Silver nitrate (AgNO<sub>3</sub>) staining of nucleolar organizer regions was performed as described before (17). Briefly, two volumes of aqueous silver nitrate 50% were mixed with one volume of gelatin 2% (prepared in formic acid 1% v/v) to make silver nitrate staining solution. The samples were stained with freshly prepared staining solution for 15 minutes at 37°C and in a dark place. After staining, the slides were rinsed with deionized water, dried, and mounted with Canada balsam (17).

### Image Analysis

A light microscope equipped with a high-resolution lens (magnification = 1000X) and a digital camera (Euromex, Holland) was used to capture the images of BM cells. All the captured images were saved as tagged image file format with the same sizes and resolutions (Figure 1). The area, length, and number of AgNORs were calculated by Scion Image Software (Version Beta 4.0.2, Scion Corporation, USA) in 100 BM cells for each rat. AA and AL were presented as pixels ranging from 0 (for white) to 255 (for black). Then, the mean of AA, AL, and AN was computed for each group (17,18).

### Statistical Analysis

The results are presented as a mean  $\pm$  standard deviation. A one-way analysis of variance (ANOVA) test was used to



**Figure 1.** AgNOR Patterns in Bone Marrow Cells. Note. AgNOR: Argyrophilic nucleolar organizer regions. The cytoplasm of the cells is unclear. The nuclei of cells are observed with different sizes and pale-red colors. AgNORs appear as small black and dark brown intranuclear dots in the cells. Some cells contain more than one AgNORs (Magnification = 1000X).

compare the mean of AA, AL, and AN among the groups. Further, the least significant difference (LSD) test was applied to compare the AA, AL, and AN between the two groups. A regression analysis was performed to calculate the relationship between the densities and the amounts of AA, AL, and AN. A  $P < 0.05$  was considered significant, and finally, SPSS (version 16) was used for data analysis.

## Results

During the exposure period, two rats died from 1 and 500  $\mu\text{T}$  groups. The one-way ANOVA analysis of AA, AL, and AN data demonstrated statistically significant differences among the groups. The analysis of complementary LSD test between each two groups demonstrated that the exposure of rats to 1  $\mu\text{T}$  of 50-Hz MFs caused a reduction in the mean of AA ( $21.5 \pm 6 \times 10^3$  pixels), AL ( $8.6 \pm 2 \times 10^3$  pixels), and AN ( $238.6 \pm 5$ ) compared with AA ( $27.4 \pm 5 \times 10^3$  pixels,  $P = 0.003$ ), AL ( $10.3 \pm 1 \times 10^3$  pixels,  $P = 0.029$ ), and AN ( $288.9 \pm 5$ ,  $P = 0.013$ ) in 2000  $\mu\text{T}$ , as well as AA ( $26.0 \pm 5 \times 10^3$  pixels,  $P = 0.024$ ), AL ( $10.8 \pm 2 \times 10^3$  pixels,  $P = 0.005$ ), and AN ( $297.7 \pm 5$ ,  $P = 0.004$ ) in the control group (Table 1). The statistical analysis of data did not support such differences in AA, AL, and AN in 100, 500, and 2000  $\mu\text{T}$  groups.

Regression analysis also showed that the amount of the AgNOR index (i.e., AA, AL, and AN) sharply decreased in the 1  $\mu\text{T}$  group. However, the effect was neutralized and reached the level of control in 2000  $\mu\text{T}$  (Figure 2) by an increase in magnetic flux density. Therefore, it seems that exposure of rats to the magnetic flux density of 1  $\mu\text{T}$  of 50-Hz MFs suppresses the proliferation of BM cells. However, magnetic flux densities of 100, 500, and 2000  $\mu\text{T}$  did not affect AgNORs compared to controls.

## Discussion

The results of this study revealed that exposure of rats to 50-Hz MFs with different magnetic flux densities (i.e., 1,

100, 500, and 2000  $\mu\text{T}$ ) two hours a day for two months only decreased AA, AL, and AN in the group of 1  $\mu\text{T}$ .

AA, AL, and AN, which are indicators of the size and number of AgNORs, sharply decreased in 1  $\mu\text{T}$  and then increased at magnetic flux densities of 100 to 2000  $\mu\text{T}$  and reached the control level, representing that the BM proliferation is inhibited in 1  $\mu\text{T}$ . There are some *in vivo* and *in vitro* studies about the effect of MFs on the AgNORs and proliferation of cells (3,4,7,11). Similarly, Okudan et al investigated the effect of ELF-MFs on the AgNORs in 120 Swiss albino mice. They exposed mice to 1, 2, 3, 4, and 5  $\mu\text{T}$  of 50-Hz MFs for 40 days. Based on the results, a significant decline was observed in the AgNORs of the 1  $\mu\text{T}$  group while there was no statistically significant difference in the groups that were exposed to higher magnetic flux densities compared with the controls (7). In another *in vivo* study, Erdal et al exposed Wistar rats to 50-Hz -ELF-MFs with 1000  $\mu\text{T}$  magnetic flux density for four hours or 45 days and found a significant decrease in the MI of BM cells in both exposed groups compared with the controls (11).

Likewise, Park et al performed another experiment with similar results to this work and concluded that the effects of ELF-MFs on cell proliferation were inversely related to magnetic flux density, and the impact could be neutralized in the upper magnetic flux density. They evaluated the *in vitro* effect of intermediate frequency (200 Hz-10 kHz) on the proliferation of BT-20. BT-20 cell cultures were exposed to a range of magnetic flux densities from 3 to 7 mT for 72 hours. They found that the cell proliferation rate decreased by about 40% due to 5-mT exposure. Nevertheless, the exposed cells to 7 mT did not reveal any reduction of proliferation (3). However, Rageh et al studied the effect of whole-body exposure of newborn rats to 50-Hz MF with a density of 500  $\mu\text{T}$  for 30 days on the MI of BM cells observed about three-fold higher MI in the BM cells of exposed animals compared with the controls (4).

According to the results of this study and some other reports, there is a reduction in the size and count of AgNORs in 1  $\mu\text{T}$  of 50-Hz MFs which is closer to magnetic flux density that we may be exposed to in our ordinary life compared with 100, 500, and 2000  $\mu\text{T}$  (19). Based on the findings of Eroz et al, no change in AgNORs in higher magnetic flux density groups can be due to the protective roles of AgNOR. They reported that all living cells protect their conditions by AgNOR proteins that have protective roles in the structure and function toward dangerous agents (9).

According to some studies (20-22), a possible mechanism that may describe the difference between high and low magnetic flux densities of 50-Hz -MF on BM cell proliferation is that in higher densities, cells sense 50-Hz MFs as a robust stressor and express stress proteins such as heat shock proteins (HSPs). These proteins act as

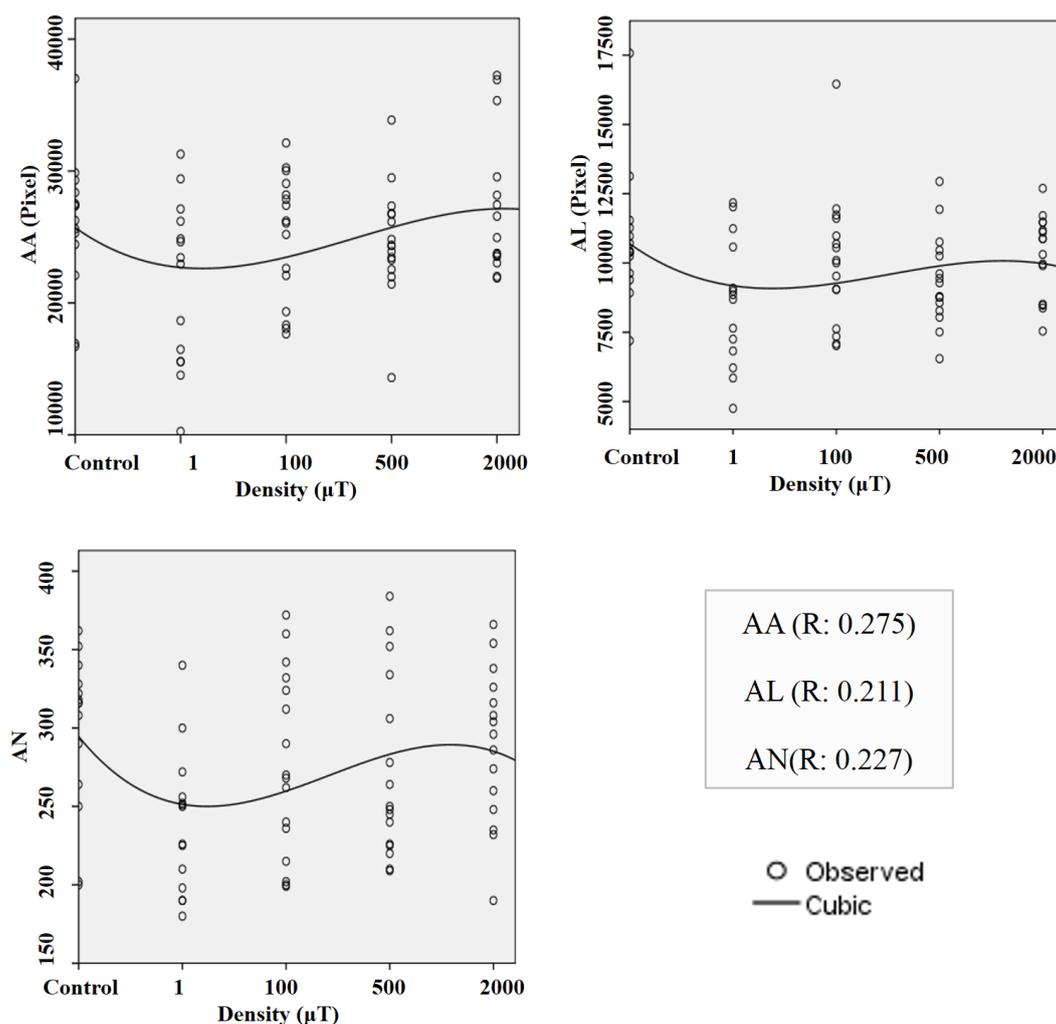
**Table 1.** Effect of 50-Hz MFs With Different Magnetic Flux Densities on AA, AL, and AN of BM Cells

Groups	No. of Rats	No. of Cells/Rat <sup>a</sup>	AA (10 <sup>3</sup> Pixels)	AL (10 <sup>3</sup> Pixels)	AN (No.)
Control	16	100	26.0 ± 5	10.8 ± 2	297.7 ± 5
1 $\mu\text{T}$	15	100	21.5 ± 6 <sup>b</sup>	8.6 ± 2	238.6 ± 5
100 $\mu\text{T}$	16	100	25.0 ± 5	10.1 ± 2	276.5 ± 6
500 $\mu\text{T}$	15	100	24.7 ± 4	9.4 ± 1	272.1 ± 6
2000 $\mu\text{T}$	16	100	27.4 ± 5	10.3 ± 1	288.9 ± 5
P value <sup>c</sup>	--	--	0.048	0.046	0.045

Note. LSD: Least significant difference; ANOVA: Analysis of variance; BM: Bone marrow; AgNOR: Argyrophilic nucleolar organizer region.

<sup>a</sup> AgNORs were measured in 100 analyzable nuclear images of BM cells for each rat. Then, the mean of AA, AL, and AN were calculated for each group.

<sup>b</sup> Results are presented as the mean ± standard deviation. <sup>c</sup> One-way ANOVA test was used to compare the mean of AA, AL, and AN among all experimental groups. LSD test was also applied to compare AA, AL, and AN between the two groups. P values < 0.05 in statistical analyses were considered significant.



**Figure 2.** Correlation of AA, AL and, AN with an increase in the magnetic flux densities of 50-Hz MFs. Note. MF: Magnetic fields. A curve estimation analysis was performed to evaluate the correlation of AA, AL, and AN with the increase of the magnetic flux densities from 1 to 100, 500, and 2000  $\mu\text{T}$ . There were regression correlations (cubic model) among AA, AL, and AN with the magnetic flux densities.

molecular chaperones and protect the cells from apoptosis. For example, HSP70 is one of the powerful anti-apoptotic proteins that can be expressed during stress.

In one *in vitro* experiment, exposure of murine femoral BM cells to 50-Hz MFs with a magnetic flux density of 80 mT showed suppression in cell division and differentiation of granulocyte-macrophage progenitors. Therefore, it can be proposed that hemopoietic cells might be a target for the detrimental effect of 50-Hz MFs (23). In another *in vitro* study, Huang et al attempted to determine the molecular mechanism of cell division inhibition by ELF-MF. To this end, they exposed a human immortalized epidermal keratinocyte cell line to a magnetic flux density of 1.5 mT of 60-Hz MF for 144 hours. Protein expression analysis demonstrated a G1 phase arrest of the cells, an increased activity of the ATM/Chk2 signaling, and a higher level of p21 protein (6).

Accordingly, 50-Hz MFs decrease AgNORs at the magnetic flux density of 1  $\mu\text{T}$ , which may represent the inhibition of proliferation in BM cells. However, 50-Hz

MFs with higher magnetic flux densities of 100, 500, and 2000  $\mu\text{T}$  did not affect the AgNORs and proliferation of BM cells. Thus, the impact of 50-Hz MFs on BM cell proliferation could be inversely associated with the density of the magnetic field.

#### Authors' Contributions

Conceptualization: AZ, MA, and MR; Method: AZ, MA, and MR; Software: AZ; Validation: AZ, MYA, and SN; Formal Analysis: AZ, and MR; Investigation: MR; Resources: AZ; Data Curation: AZ and SN; Writing-Original Draft Preparation: AZ, and MR; Writing-Review & Editing, AZ, and SN; Visualization: AZ; Supervision: AZ and MYA; Project Administration: AZ and MR; Funding Acquisition: AZ.

#### Conflict of Interests

The authors report no conflict of interests.

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