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Exposure of Extremely-Low Frequency (ELF) Magnetic Field May Cause Human Cancer

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ABSTRACT

Introduction: Chronic exposure of non-ionizing extremely low-frequency magnetic fields (ELF-EMF) is considered as a health hazard due to its adverse effects on human body such as generation of any type of cancer. Stem cells are appropriate models to assess the effects of ELF-EMF on other cell lines and human beings. **Materials and methods:** Adipose tissue has been known as source of multi potent stromal human mesenchymal stem cells (MSCs) which can be obtained in less invasive method and in large amounts; so adipose-derived stem cells (ADSCs) were used in this study. Effect of ELF-EMF (intensities of 0.5 and 1 mT and 50 Hz) on proliferation rate of hADSCs was assessed in 20 and 40 min per day for 7 days. Trypan blue assay was performed to assess cell proliferation rate. **Result:** The results shown that 0.5 and 1 mT magnetic fields can promote the proliferation rate of adipose derived hMSCs according to the duration of exposure. **Conclusion:** These outcomes could approve the effect of ELF-EMF on cancer induction; although the effective mechanisms in this process are still unknown.

Key words: Electromagnetic fields, extremely low frequency, exposure, cancer,stem cell

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INTRODUCTION

The electrical devices and electrical power lines producing electromagnetic field are in our environment. Johansson has claimed that standards of ELF-EMF exposure need to be controlled because chronic ELF-EMF exposure has potential adverse effect on human health.[1] Some scientists thought that current exposure safety limits are insufficient to provide public health.[2] EMF may disturb immune function via stimulation of various allergic and inflammatory responses and effects on tissue repair processes. These disturbances may cause some diseases like cancer.[1] So, a major concern about the adverse effects of non-ionizing EMF exposure is cancer induction.

Many epidemiological studies have shown that EMF can cause several kinds of human cancer.[3,4] One of the most well-known epidemiologic studies indicated that probability of blood cancer was higher in children living near the transmission electric power lines.[5] These early studies shown an important fact about effect of EMF on human health.[6] In some epidemiological studies, Tumors of the nervous system were found resulting in long-term mobile phone use (more than 10 years).[7-10] Some prior studies focused on breast cancer promotion under exposure of electromagnetic fields such as review of Caplan and his collogues in 1999.[11] In this study, results shown higher incidence of breast cancer among male electrical workers.[11]

In addition to epidemiological studies, we also demand biological studies to assess the effects of EMF on cancer creation. One of the most significant phenomena in cancer is increasing cell proliferation. Any external physical or chemical factor that causes increase in cell proliferation can also cause cancer in tissue. The results of Grasse's study in 2010 indicated that ELF exposure significantly increase neurogenesis in the dentate gyrus (DG) of adult mice[12] but in other studies, the outcomes showed that ELF-

EMF can inhibit the cell proliferation.[13] Rodriguez and his colleagues studied the effect of pulsed magnetic field (intensity of 0.65 mT and 4 Hz) on human osteoblasts for 45 min, results showed no change in cell number.[14] Another study that conducted on osteoblast proliferation showed ELF-EMF with frequency of 50 Hz with intensities of 0.9-4.8 mT for 30 min per day can inhibit cell proliferation.[15]

One of the main reasons for this disparity in results is different cell line. Different cells have dissimilar sensitivity, cell cycle time and response to extrinsic factors. So, we have got to use a special cell type which can be a reference for other cell lines. Stem cells, undifferentiated cells, with high renewable capacity are good choice for this purpose.[16] Furthermore, there are a lot of Adipose tissues in human body that are sources of mesenchymal stem cells; therefore, we decided to investigate whether the magnetic field has any impact on proliferation rate of these cells.

In this study, we assessed proliferation rate of hADSCs in two flux densities of 0.5 and 1 mT in duration of 20 and 40 min per day for 7 days considering this effect can be cancer induction.

MATERIALS AND METHODS

Magnetic field exposure system

A continues sinusoidal 50Hz magnetic field was made by a solenoid coil which was wound with 720 turns of 1 mm enamel copper wire on a cylindrical core of acrylic tube (inner diameter: 20 cm, height: 24 cm) and connected to an autotransformer serially. The input sinusoidal signals to solenoid were evaluated by oscilloscope. Favorite flux density of magnetic field was obtained via setting voltage percentage scale on autotransformer and electric current and voltage for generating magnetic flux density were assessed by digital multimeter (digital HiTESTER.3256-50, Japan). Calibration of system was performed via teslameter (LEYBOLD DIDACTIC GMBH 51662, Germany) and its probe AXIALE B-SONDE (model: 516.61).[17] The temperature in solenoid during ELF-EMF exposure was controlled through a digital thermometer (Digital Hygro-Thermometer, France).

Isolation and culture of hADSCs

Adipose tissues were used from three women with age of 23-41 years old. Briefly, samples were cleaned with sterile PBS to remove debris and red blood cells; Then, 0.075% collagenase type I in PBS was added to aspirates for 30 min at 37^{0}C with gentle shaking. The collagenase I was removed with DMEM/10% FBS and the infranatant was centrifuged for 10 min at 750 rpm. The cellular pellet was re-suspended in DMEM/10% and FBS and plated in T_{25} flasks in 5 ml DMEM+F12 medium and 10% FBS and 1% antibiotic. The non-adherent cells were discarded and adherent cells were washed twice with PBS after 24 h. Cells were passaged with Trypsin/EDTA harvesting, when the confluency of cells was reached to 80-90% in culture flask. After two passages, hMSCs were plated in 96-well plates and used to experiment. The cultures were kept at a temperature of 37^{0}C in humid atmosphere with %5 CO2. The medium was changed every 3 days. In previous study, we determined isolated cells in this way are stem.[18]

Exposure protocols

Human ADSCs were cultured in 96-well plate at the density of 10^3 cells per well and incubated overnight. The plates were exposed to the 50 Hz EMF with intensities of 0.5 and 1 mT for 20 and 40 min per day for 7 days. Exposure groups were: 1mT for 40 min, 1mT for 20 min, 0.5 mT for 40 min and 0.5 mT for 20 min per day. The control groups were placed in solenoid off-coils for 20 and 40 min per day, so all Conditions were equal for exposure and control groups. In all experiments, cell plates were located at the center of solenoid where the magnetic field was uniform.

Trepan blue assay

Proliferation rate of hADSCs after exposure of ELF-EMF was determined via trypan blue assay. After treatments, cells were washed with PBS and harvested by trypsin/EDTA for few minutes, then centrifuged and re-suspended in a solution of trypan blue1:2 in PBS; finally, cells were counted with a Neubauer chamber and phase contrast microscope.[19] Proliferation rate was calculated with below equation:

proliferation rate=
(the number of living cell)/(initial total cells)×100

Statistical analysis

One way ANOVA was used to assessment the significant differences among groups. Differences were significant when *P<0.05. All statistical analysis was performed with the SPSS 19 software.

RESULTS

Magnetic field exposure system

Autotransformer and voltage percent scale, was connected to solenoid (Figure 1). The sinusoidal signals at the first of experiment was assessed by the oscilloscope connected to the solenoid (Figure 2). Through setting the voltage percentage scale, the favorite magnetic flux density was generated in solenoid (Table 1).

Table 1

	0.5 mT	1mT
Current(mA)	202.1	4.68
Voltage(V)	395	9.19

Figure 1: The Autotransformer.

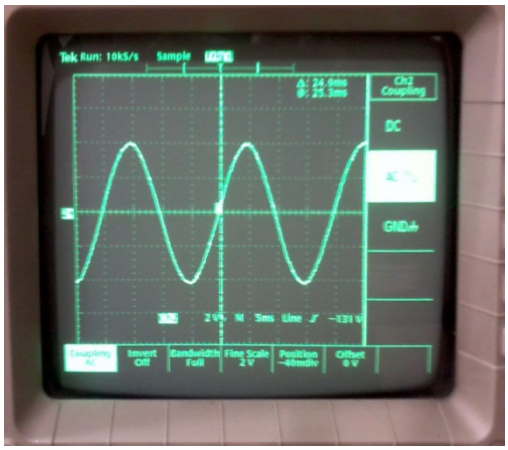

Figure 2: The oscilloscope.

Figure 3: Teslameter and its probe

Figure 4: Homemade solenoid coils with calibrated plate. Digital thermometer probe located at the center of solenoid.

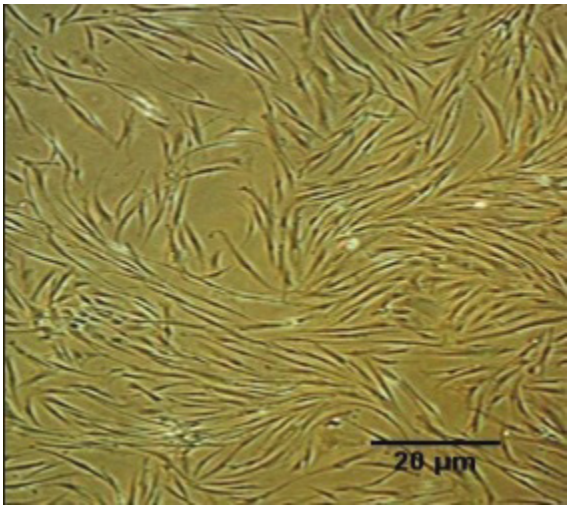

Figure 5: Spindle- like morphology of ADSCs.

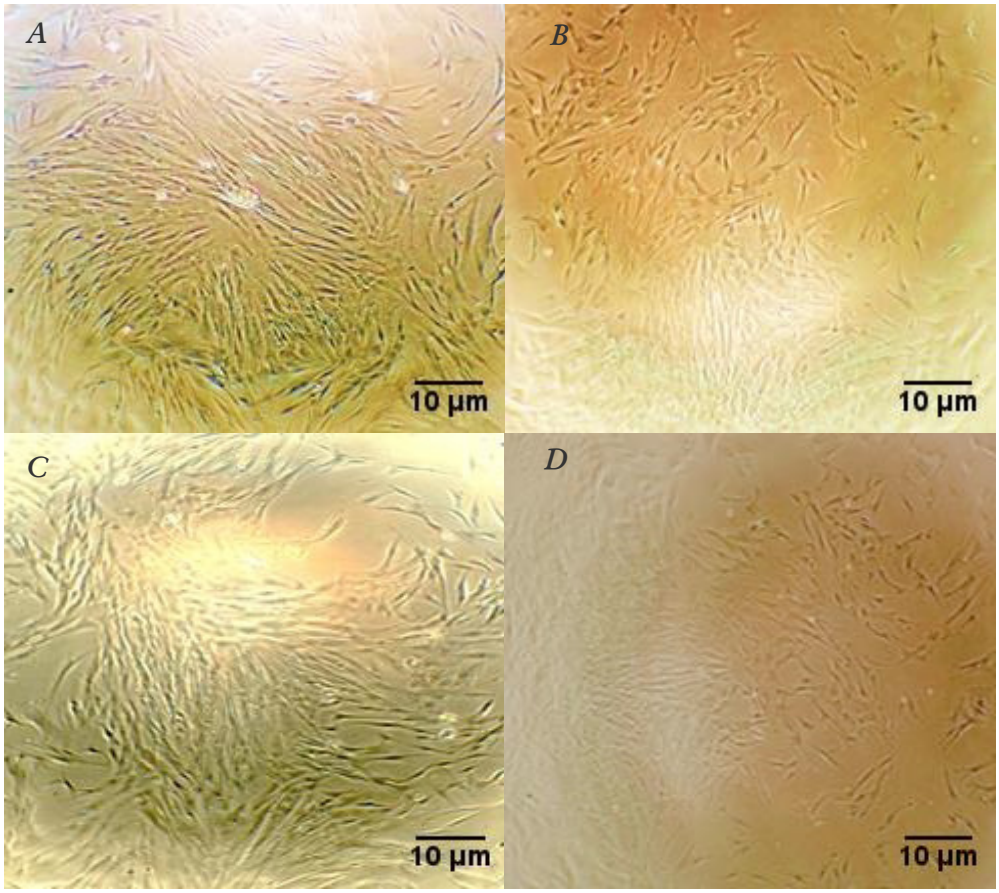

Figure 6: **The morphological assessments of hADSCs showed orientation of cells exposed to magnetic field versus alignment of non-exposed cells (control).**
A) Exposure (40 min), B) Control (40 min), C) Exposure (20 min), D) Control (20 min).

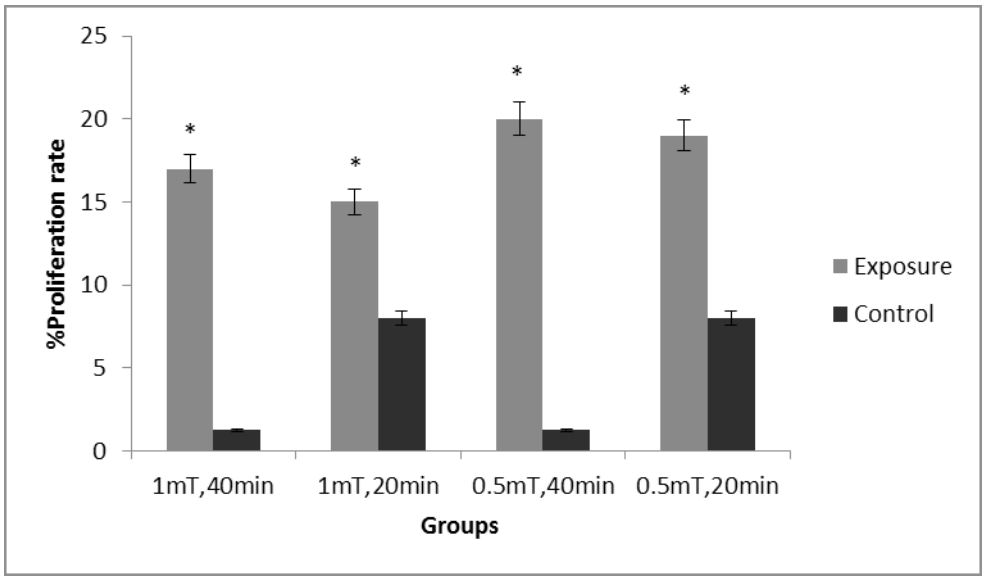

Figure 7: **Proliferation rate in exposure and control groups. The proliferation rate in exposure groups was significantly higher than that in control groups (P<0.005*).**

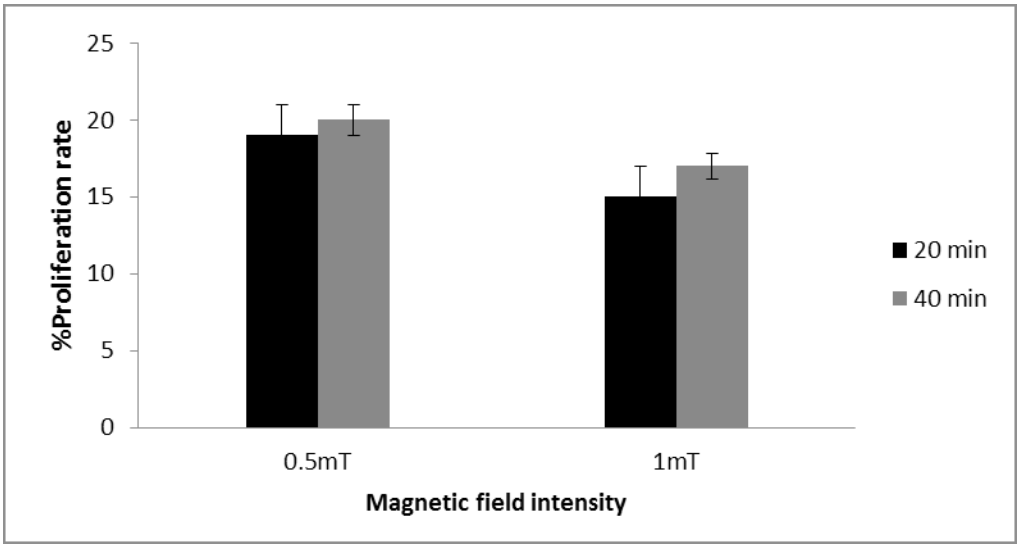

Figure 8: Effect of exposure time on proliferation rate in both exposure groups. Proliferation rate in 40 min exposure was higher than that in 20 min exposure (P<0.005*).

Exposure system Calibration

The exposure system was calibrated using teslameter (Figure 3). The uniformity of magnetic field was measured in different distance from center of solenoid and the different uniformity areas were marked on a plate inside the solenoid coils. Cells were placed in the central of solenoid with most uniformity in all experiments (Figure 4).

Temperature control

Digital thermometer probe placed inside the solenoid indicating magnetic field-induced heating. Induced heating was ignorable during exposure(less than %1) (Figure 4).

Isolation and culture of hADSCs

Adipose derived stem cells morphology was assessed after one week by means of phase contrast microscope. The ADSCs had fibroblastic and spindle- like morphology (Figure 5).

Morphological assessment of hADSCs exposed to ELF-EMF

The morphological assessments by phase contrast microscope showed that hADSCs exposed to ELF-EMF (1, 0.5 mT) were oriented together to a common vector despite the unexposed cells (Figure 6). This morphological assessment was prepared after 7 day EMF exposure.

Proliferation rate of hADSCs

Cell proliferation rate was investigated via trypan blue staining. Proliferation rate significantly rose in all exposure groups. Generally, proliferation rate of hADSCs in magnetic field of 0.5 mT was more than that in 1 mT (Figure 7) and it increased with increasing the magnetic exposure time in both magnetic field intensities (Figure 8).

DISCUSSION

In this study, we assessed 20 and 40 min per day exposure of 50 Hz (0.5 and 1 mT) ELF-EMF on proliferation rate of hADSCs for 7 days. Proliferation rate in all exposed groups was significantly higher than unexposed (control) groups.

There was no linear correlation between magnetic field intensity and proliferation rate; According to trypan blue results, the amount of proliferation in 0.5 mT group (20 and 40 min) was higher than 1 mT group (20 and 40 min). It means that magnetic intensity of 0.5 mT had more effect on stem cells proliferation rate. This impact has been known as "window effect" in literature.[20-22] In many studies "magnetic dose" has proposed as magnetic field intensity multiplied by exposure time;[23,24] According to definition of magnetic dose, in groups of 0.5 mT in 40 min and 1 mT in 20 min, cells received the same magnetic dose. Our results revealed that the proliferation response of hADSCs was not the same to the similar magnetic dose.

Two flux densities (0.5 and 1 mT) were applied in our study, because magnetic flux density can be between 0.1 and 1 mT nearby electrical devices. According to international radiation protection association [IRPA/INIRC, 1990] guidelines, Intensities of 0.5 mT and 5 mT were selected as safe limitation of work day exposure and short period (2 h) exposure respectively.[3] It is shown that DNA single-strand breaks can occur in 0.1 mT magnetic fields in rat brain cells and double–strand breaks increase in 0.5 mT and 1mT, While these intensities are under the permissible limit of IRPA/INIRC and NRPB.[3]

Study of Lai *et al*. showed that 2 hour exposure of 60Hz magnetic field (with flux densities of 0.1, 0.25, and 0.5 mT) trigger DNA single and double-strand breaks dose-dependently in animal brain cells. DNA strand breaks may cause some disruptions in cellular functions, initiate cell death, and induce neurodegenerative disease.[3] Sometimes cells with strand

breaks stay alive and continue their uncontrolled proliferation; in this situation this unhealthy cell becomes cancerous cells. In previous studies had been shown that ELF-EMF with 0.5 and 1 mT can cause DNA strand breaks in cells, so we decided to investigate whether these intensities could make cell proliferation to increase or not.

The effects of ELF-EMF on cell proliferation often depends on cell type [25,26] as Van Den Heuvel *et al*. showed that stromal stem cell proliferation (CFU-f) reduced in female mice exposed to ELF-EMF, while CFU-f proliferation did not decrease in male mice. [13]Another study showed a slight temporary reduction in the proliferation rate after 1 mT ELF-EMF exposure. Their results demonstrated that undifferentiated and differentiating PC12 cells had more sensitivity to ELF-EMF while the fully differentiated cells were less sensitive and more stable.[25] According to these results, it seems that stem cells could be suitable candidate to investigate the effect of ELF-EMF on human health.

The mechanisms of EMF effects are still unknown.[27,28] One of the possible mechanism is an enzyme which establishes normal Na-K slopes across the cell membrane; Magnetic field can cause electron moving with velocity of 10^3 m/s cross this enzyme.[29] Simko and Mattsson suggested that increase of free radicals could be transducer of ELF-EMF effects in cell.[30] Other studies shown that low energy EMF (ELF) produces stress responses via interacting with electrons in DNA molecules directly increasing may cause some breaks in DNA strands.[2] study of Liburdy *et al*. indicated that ELF magnetic fields can boost breast cancer cell proliferation via disruption of melatonin's natural oncostatic action.[31] Joan *et al* shown that 1.2 μT, 60 Hz magnetic fields meaningfully block growth inhibitory action of pharmacological levels of tamoxifen (10^{-7} M) on MCF-7 cells.[32]

CONCLUSION

According to our investigation, ELF-EMF can cause increase in hADSCs proliferation rate and if we consider the probability of DNA strand breaks simultaneously, it can lead cells to cancerous state. A cancer cell has uncontrolled proliferation with some DNA strand breaks damages occurred in chemical and physical conditions. Furthermore, exposure of ELF–EMF can obstruct the effect of growth inhibitors hormones and help breast cancer developing. Maybe this phenomenon induces some cancer such as breast cancer.

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CONFLICT OF INTEREST

No conflict of interest

ABBREVIATIONS USED

EMF:Electromagnetic field ; ELF:Extremely low frequency ; hADSCs:human adipose-derived stem cells ; MSCs:Mesenchymal stem cells ; PBS: Phosphate-buffered saline ; FBS:Fetal bovine serum

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