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Original Article

Environmental 50Hz Magnetic Fields Can Increase Viability of Human Umbilical Vein Endothelial Cells (HUVEC)

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Abstract

Introduction
Over the last decades, considerable levels of electromagnetic fields (EMFs) have been characterized in the living environment. Recent epidemiological studies on occupational and residential exposure to EMF have shown that 50/60 Hz fields, known as extremely low frequencies (ELF), have various biological effects, such as angiogenesis. This study aimed to investigate the effect of environmental 50 Hz magnetic fields at intensities of 3, 6, 15, 46, 110 and 207 μT on human umbilical vein endothelial cells (HUVECs) as a significant component of angiogenesis process.

Materials and Methods
In this study, 43 experimental groups were evaluated, including a control group, 6 sham exposure groups, 18 acute, and 18 chronic exposure groups with different exposure intensities and durations of exposure. Proliferation and viability of HUVECs were examined via cell counting and MTT methods, respectively.

Results
No significant changes were observed in the proliferation of HUVECs by 50 Hz magnetic field, while the viability of some acute groups was found to increase. These findings confirmed the theory of "biological window" for magnetic fields.

Conclusion
According to the results of this study, since the 50 Hz magnetic field can effect on viability of HUVECs and these cells play a key role in angiogenesis, 50 Hz magnetic fields at the mentioned intensities probably could be effective in the improvement of angiogenesis process.

Keywords: Magnetic Field, Endothelial Cells, Viability, Proliferation

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1. Introduction

Over the last decades, considerable levels of electromagnetic fields (EMFs) have been characterized in the living environment. EMFs with frequency ranges of 0-300 Hz are known as extremely low frequencies (ELF). The most important frequencies of ELF fields are 50/60 Hz, which are produced wherever electrical current flows in power lines and cables, residential wiring and electrical appliances [1-4]. Recent epidemiological studies on occupational and residential exposure to EMF have shown that 50/60 Hz fields have various biological effects [2-11].

According to the literature, ELF-EMFs can cause some biological effects such as reducing the bone fracture healing times [12], increasing in the single- and double-strand DNA breaks [1, 2, 4, 7, 8], promoting or preventing tumor growth [13-16], and accelerating the wound healing [17].

Intensity changes of environmental magnetic fields depend on time, measurement location, and available electrical appliances. In a study, Kaviani et al. investigated the effects of environmental magnetic fields on snail neurons [18], characterizing the intensities of available 50 Hz magnetic fields in the environment via dosimetry techniques. According to the results, intensities of 2.83, 6.02, 14.91, 45.87, 109.34 and 207.2 μT were the min of the minimums, mean of minimums, mean of averages, mean of maximums, max of maximums, and cumulative average of magnetic field flux, respectively. Furthermore, the researchers claimed that 50 Hz magnetic field with intensity of 6.02 μT decreased the resting potential of neurons in acute and chronic exposure conditions.

In addition to the previously mentioned studies about biological effects of ELF-EMFs, some studies have denoted that these magnetic fields influence angiogenesis [13, 16, 19, 20]. Angiogenesis is defined as the growth of new capillaries from the existing blood vessels, which is mediated by a series of cellular events leading to neovascularization [21]. Angiogenesis plays a pivotal role in various physiological processes during human fetal development, as well as tissue repair after surgery or traumatic injury, wound healing, menstrual cycle, and treatment of cancer and ischemic and inflammatory diseases [22].

Since endothelial cells are essentially involved in angiogenesis, understanding the metabolism and signaling of endothelial cells could reveal potential therapeutic targets for the inhibition or enhancement of angiogenesis [23].

Considering the effects of magnetic fields on angiogenesis as reported by previous studies [10, 13, 14, 19, 20] and deficiencies in evaluating the effectiveness of local environmental magnetic fields in angiogenesis, this study aimed to assess the effect of 50 Hz environmental magnetic field on endothelial cells (the most important cells involved in angiogenesis) in vitro. Furthermore, we studied the effects of 50 Hz magnetic fields with diametrical intensities on the proliferation and viability of endothelial cells [24].

2. Materials and Methods

2.1. Exposure system

In this study, magnetic field exposure system was a pair of Helmholtz coils, a 50 Hz power generator, and an amplifier system designed by Kaviani et al. based on dosimetry results of 50 Hz magnetic field [18].

2.2. Cell line

This study aimed to determine the effects of environmental magnetic fields on angiogenesis of cancer patients. Endothelial cells, which are the most important components of angiogenesis, were selected for the in-vitro examination of angiogenesis.

In addition, human umbilical vein endothelial cell (HUVEC) line was cultured in DMEM-F12, containing 10% fetal bovine serum and 1% pen-strep, and incubated in 5% CO2 at the temperature of 37°C.

2.3. Experimental groups

HUVECs were seeded in six central wells of a 24-well plate placed between the Helmholtz coils and exposed to 50 Hz magnetic field with intensities of 3, 6, 15, 46, 110, and 207 μT [18]. Two types of exposure (Acute and chronic induced for 10, 20, and 30 minutes only on the 5th day of plating and for 5 consecutive days, respectively.

Since the exposure was performed out of the incubator at room temperature, six sham groups were considered for each exposure duration (three acute and three chronic exposure groups) in order to diminish the effects of environmental factors (e.g., temperature and, humidity on HUVECs. For this purpose, the sham groups were placed in turned off coils.

2.4. Determination of cell proliferation

HUVECs were detached after the third passage, and 5000 cells/well were seeded in six central wells of the 24-well plate. In the treatment groups (acute and chronic exposure), the cells of three adjacent wells were separately counted using trypan blue 24 hours after the final exposure. Mean values were considered as the cell number of each plate, and cell proliferation rate in the exposure groups was compared with the control groups.

2.5. Determination of cell viability

Magnetic field cytotoxicity of HUVECs was evaluated using the MTT assay (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Invitrogen, GIBCO, USA). A new cell culture medium (300 µl/well) was added to the wells eight days after plating (62 hours after the last exposure of each group). Moreover, 30 µl of MTT solution (5 mg/ml in phosphate-buffered saline) was added to the wells. Following that, the cells were incubated at the temperature of 37°C for four hours. After yielding purple formazan crystals, the residual solution was removed, and 300 µl of dimethyl sulfoxide (DMSO) was added to each well of the 24-well plate.

After incubation (10 minutes) and shaking the plate, 200 µl of the purple solution in each well was transferred to the 96-well plate, and optical density (OD) was measured using a multiscan ELISA reader (LabSystems Multiskan MS, U.K.) with a 540-nm filter. Obtained mean values were considered as the final OD of each plate, and cell viability was reported as ratio of exposure_groups in comparison with the control group.

2.6. Statistical analysis

Obtained results were presented as three-time repetition means and depicted in bar graphs. Data analysis was performed in SPSS V.20 for windows (SPSS Inc., Polar Engineering and Consulting). All data had normal distribution. Significance of the differences between the treatment groups was determined using the analysis of variance (ANOVA) and least significant difference (LSD) test. In this study, P value of less than 0.05 was considered statistically significant.

3. Results

In order to determine the primary effects of acute and chronic exposure of environmental 50 Hz magnetic fields on angiogenesis, proliferation and viability of HUVECs were evaluated at various intensities (3, 6, 15, 46, 110 and 207 µT) and exposure durations (10, 20 and 30 minutes). Results of different evaluations are presented in this section separately. Moreover, results of acute and chronic exposure were compared with the same duration and at different intensities.

3.1. Effects of acute and chronic exposure of 50 Hz magnetic field on HUVEC proliferation

Number of cells was normalized by the number of cells of control group and reported as normalized values (Figure 1). According to our findings, none of the protocols had a significant effect on HUVEC proliferation.

3.2. Effects of acute and chronic exposure of 50 Hz magnetic field on HUVEC viability

Viability of HUVECs was measured via the MTT assay, and the results were reported as the normalized value of the mean OD (caused by dissolved purple formazan crystals in DMSO) in exposure groups compared to the control groups (Figure 2).

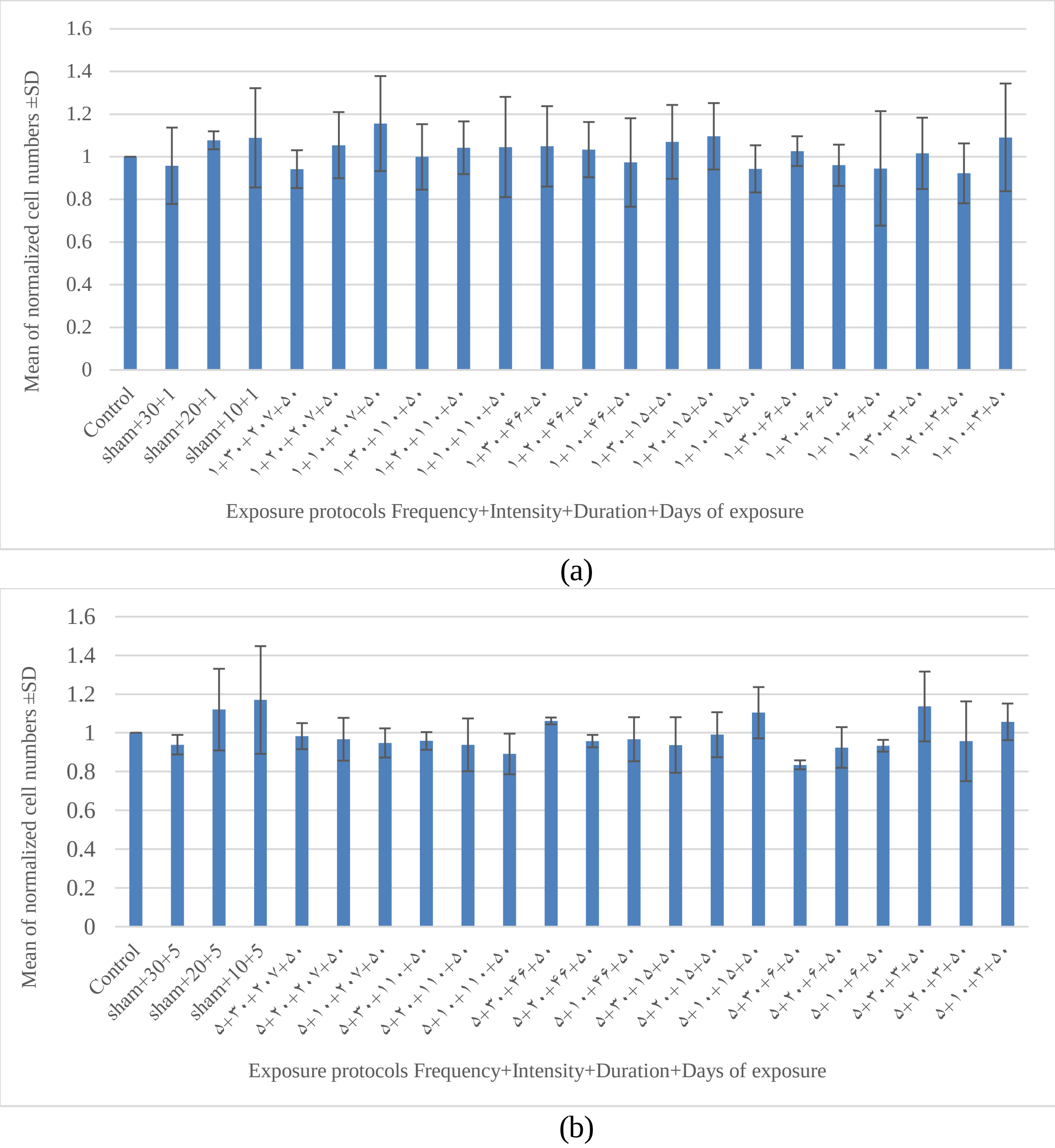


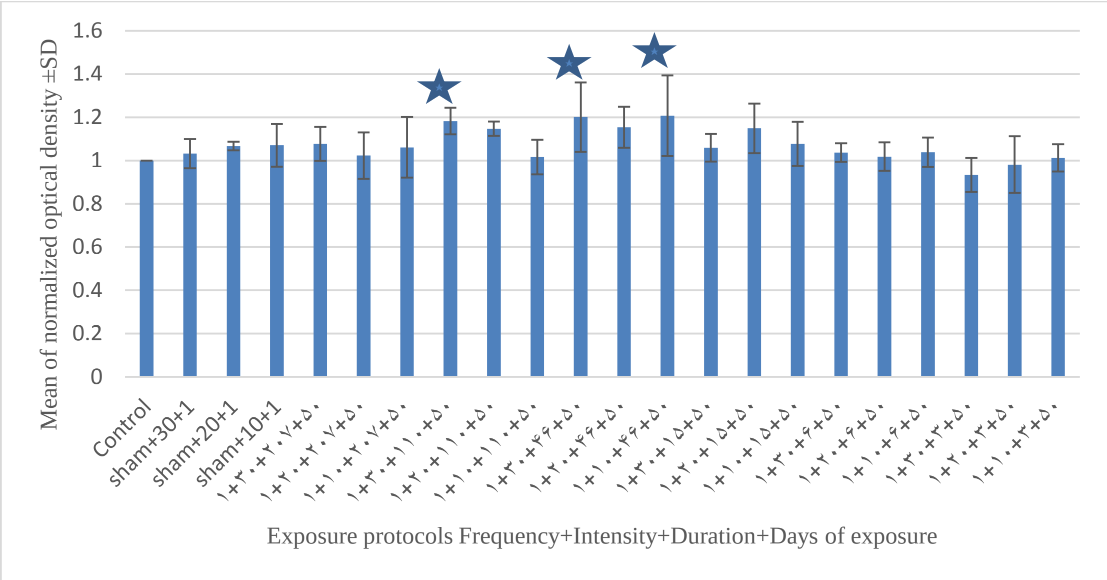
Figure 1. Normalized number of HUVECs in acute (a) and chronic (b) exposure of 50 Hz magnetic field with various intensities and exposure durations (mean±SD)

In acute exposure conditions, the following protocols significantly increased the viability of HUVECs: 110 µT (30 minutes), 46 µT (30 minutes), and 46 µT (10 minutes). Moreover, protocols of 110 µT (30 minutes) and 46 µT (30 minutes) increased the viability of HUVECs in chronic exposures conditions.

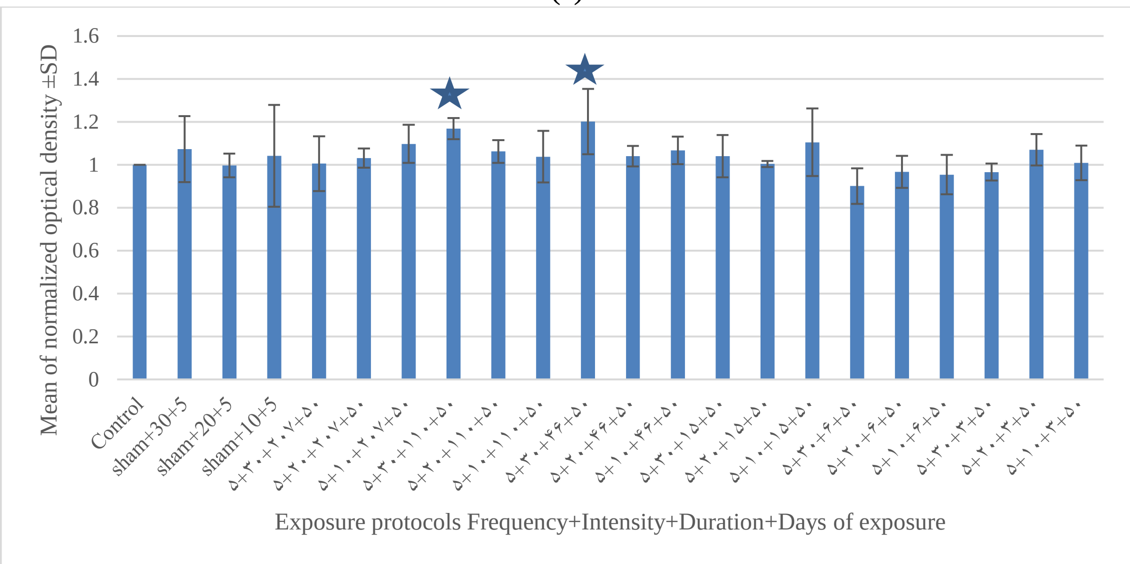
Effective protocols of 50 Hz magnetic field in the proliferation and viability of HUVECs are shown in Table 1.

3.3. Effects of chronic and acute exposure on HUVECs

For the statistical analysis of the differences between acute and chronic exposure, an orthogonal comparison was carried out between contrasting acute versus chronic exposure groups, the results of which are depicted in Figure 3 (normalized ODs expressed as mean±SD). According to the information in this figure, acute exposure was more effective on viability of HUVECs compared to chronic exposure ($P<0.1$).



(a)



(b)

Figure 2. Normalized optical densities of HUVECs in acute (a) and chronic (b) exposure of 50 Hz magnetic field with various intensities (μT) and exposure durations (mean±SD) (*P<0.05, significantly effective protocols for HUVEC viability)

Table 1. Effective protocols of 50 Hz magnetic field in HUVEC proliferation and viability

Type of Exposure / Exposure Duration	Acute Exposure	Chronic Exposure
10 min	46 μT	----
30 min	46 μT	46 μT
30 min	110 μT	110 μT

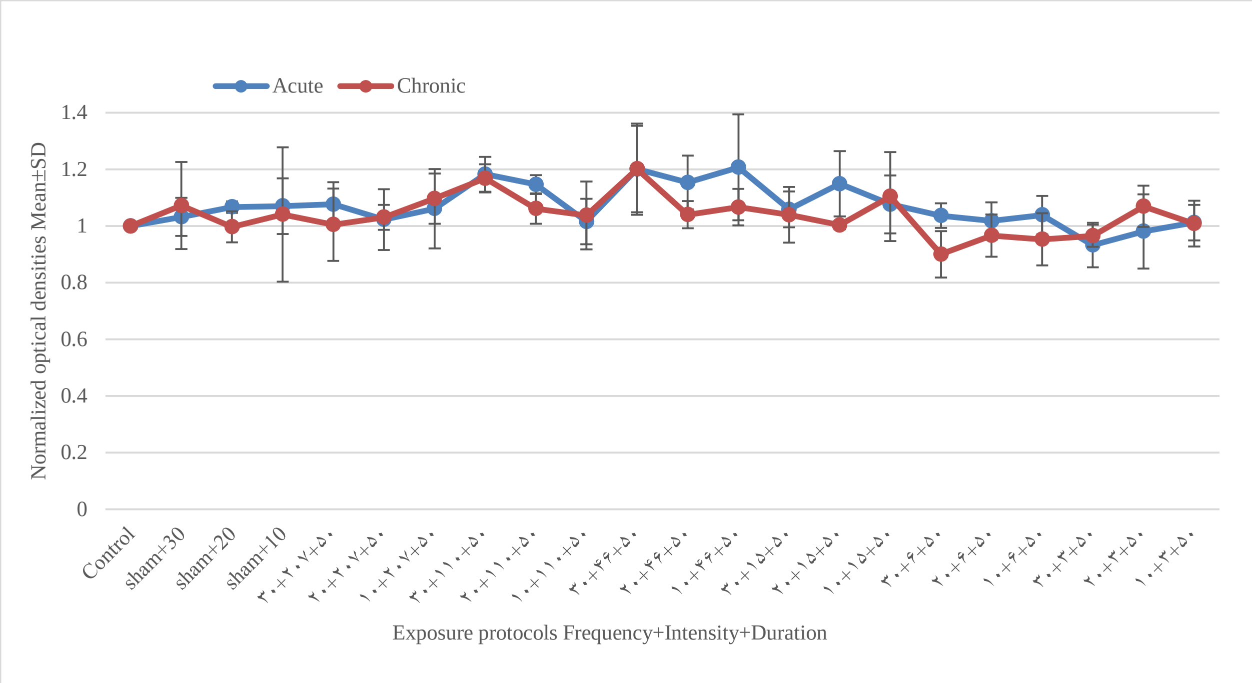


Figure 3. Normalized optical densities of acute and chronic exposure of 50 Hz magnetic field exposure on HUVECs (mean±SD)

4. Discussion

According to the literature, EMFs change cell functions and induce different responses in living organisms. Such examples are the effects of EMFs on cell proliferation and differentiation, cell cycle disorder, intracellular interactions, induction of programmed cell death, DNA replication, gene expression, cellular communications, and free radical production [25]. Furthermore, low-frequency EMFs have been applied in the treatment of specific pathological conditions, including bone fractures, skin ulcers, and migraines [26].

In the present study, investigation of the effects of acute and chronic exposure of sinusoidal magnetic field (50 Hz with intensities of 3, 6, 15, 46, 110, and 207 µT) on HUVECs indicated that 110 and 46 µT magnetic fields could significantly increase cell viability in vitro. This finding is inconsistent with the results obtained by Williams et al., which showed that 10, 15, and 20 mT EMFs (100 pulses/sec rectified pulse shape) reduced angiogenesis in breast adenocarcinoma [13]. This discrepancy could be due to magnetic field shapes, sine and rectified sine wave magnetic fields or application of different intensities.

Another study in this regard was conducted in 2014 on the optimization of therapeutic EMFs to retard breast cancer tumor growth and vascularity. According to the findings, EMF exposure at 10 mT significantly suppressed tumor growth compared to the control group. However, the researchers reported that increased treatment duration using a constant intensity caused no additional tumor mass suppression. Moreover, the results suggested that increased intensity of 10-20 mT led to a dose-responsive tumor suppression compared to the control group [16].

In another research, Okano et al. stated that static magnetic field affects tubular formation in vitro, depending on the type of cells and conditions of exposure (e.g., intensity, localization, duration and heterogeneity or homogeneity of magnetic fields) [27].

Furthermore, Monache et al. conducted two studies to assess the effects of 50 Hz magnetic field on angiogenesis and endothelial cells. Initially, it was indicated that some important angiogenic functions of endothelial cells in vitro (e.g., proliferation, migration and tube formation) increased due to the influence of sinusoidal EMF (1 mT, 50 Hz) [10]. On the other hand, the researchers claimed that EMFs with similar parameters at a higher intensity (2 mT, 50 Hz) induced a significant reduction in angiogenesis [28].

This incongruity between the two protocols (1 mT, 50 Hz and 2 mT, 50 Hz) could be explained with the “biological window” theory. Consistent with these results, we observed that some intensities of 50 Hz magnetic field (46 μT and 110 μT) have a significant effect on the viability of HUVECs.

These findings could be explained with the “biological window” of magnetic field effects, particularly the “amplitude window” hypothesis [29]. According to this theory, suggested existence of “specific permitted” levels, which could be attained by biosystems under the influence of magnetic fields, is associated with the metabolic status of the system. Moreover, the response of biological systems in different metabolic conditions yields variable results [30].

Contrary to the findings of the aforementioned studies, Cameron et al. stated that raising the intensity of exposure from 10 or 15 to 20 mT for 10 minutes increased the tumor-doubling time dose-responsively [16].

According to the results of the current study, increasing the exposure duration at each intensity (from 10 to 20 to 30 minutes) once a day or for five consecutive days in chronic exposure conditions did not amplify the effects on HUVECs significantly. This is in line with the results of the studies by Cameron et al. and Tatarov et al., who demonstrated that increased daily exposure time caused no significant suppression of tumor growth and vascularity, respectively [15, 16].

As observed in the present study, acute ELF magnetic field exposure is more effective on viability of HUVECs compared to chronic exposure. This is inconsistent with the findings of Kaviani et al., which denoted that chronic exposure of 50 Hz magnetic field has more stable effects on the reduction of neuronal spike frequency in snail neuron cells [24]. This discrepancy could be due to the type of the exposed cells.

One of the clinical applications of EMFs is the acceleration of bone fracture healing [12], the main mechanism of which remains unclear. According to our data on HUVECs, environmental 50 Hz magnetic fields are presumably able to promote angiogenesis. This suggests that magnetic fields may facilitate healing by joining osteogenesis and blood vessel growth processes. Moreover, this finding emphasizes the protective aspects of magnetic field application, so that increased angiogenesis induced by environmental magnetic fields could adversely affect cancer patients. Therefore, these patients must avoid industrial regions and using electronic devices as far as possible.

Reoxygenation is one of the 4 R’s of radiobiology, which influences the sensitivity of cancer cells to ionizing radiation. During radiotherapy, when the over layer of cancer cells dies due to radiation, the hypoxic inner layer of tumor cells shows radiation resistance. As such, the dose-fractionation method could be used to yield more oxygen and raise the radiation sensitivity of these cells [31].

Findings of the present study indicated that 50 Hz magnetic field at specific intensities increases angiogenesis in vitro. In cancer patients with magnetic field exposure after the first radiotherapy session, increased angiogenesis could facilitate cellular reoxygenation in the second session of radiation, enhancing the velocity and efficacy of treatment, probably.

5. Conclusion

According to the results of this study, both acute and chronic exposure of some environmental 50 Hz magnetic fields increased HUVEC viability. This specific manner (not the dose-responsive manner) of 50 Hz magnetic fields on biological systems is supported by the theory of “biological windows”.

Similar studies should be performed on cancerous endothelial cells instead of normal HUVECs. However, since the extraction of cancerous endothelial cells from tumors and preparation of a pure cell population is technically impossible, we had to use HUVE cells in our study. For future studies in this regard, it is recommended that cancerous endothelial cells be exploited rather than HUVECs in order to assess angiogenesis in tumors, which was the main objective of this study. Another limitation of our study was that we focused on the magnetic field intensities determined by Kaviani et al. (2008), which were selected via dosimetry of environmental 50 Hz magnetic fields in Tehran, Iran. However, since 2008, environmental fields have changed due to the increased number of electric appliances, power lines, and cables. Therefore, more recent data obtained by the latest dosimetry techniques are required so as to yield accurate and reliable results.

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