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The expression level of *c-myc* oncogene affected by exposure to low frequency electromagnetic fields (LF-EMFs) in mouse mesenchymal stem cells (MSCs)

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ABSTRACT

Electromagnetic fields which can be generated from homes and workplaces appliances can lead to alterations in oncogenes which cause cancer diseases in human such as breast cancer, leukemia, nervous system tumors and lymphoma. The aim of our study is to investigate the effect of LF-EMFs (50 Hz) on *c-myc* oncogene expression level in mouse mesenchymal stem cells (MSCs) by exposure to AC electromagnetic flux density: 0.38mT, 0.82mT, 1.25mT, 1.66mT, 2.13mT, 2.52mT, 2.79mT, 3.06mT, 3.71mT, 3.96mT, 4.08mT and 4.52mT using exposure unit. After 4 days of exposure, when initial changes in cell viability, morphology and count between exposed and unexposed cells were noted, total RNA was extracted to evaluate the expression level of *c-myc* oncogene by quantitative real time PCR. Results indicated that *c-myc* oncogene expression level began to increase with value 3.61% at 0.38mT, reaching to the maximum value 95.9% at 4.52mT in day 4. After 14 days of LF-EMFs exposure, *c-myc* gene expression level was 29.9% at 0.38mT and increased to reach the maximum value 98.6% at 4.52mT. These findings reveal that the effect of LF-EMFs on oncogenes expression level is very significant and deserves attention, causing harmful effects on human health and population.

KEYWORDS

LF-EMFs;
c-myc oncogene;
MSCs;
EL4-Lymphoma;
qRT-PCR.

INTRODUCTION

Electromagnetic fields (EMFs) are the force field generated around an electric current and invisible to the human eye. Common sources of electromagnetic fields include power lines, household electrical wiring, motor-driven instruments, computer screens, telecommunications and broadcasting facilities, and mobile telephones. These EMFs were initially considered too weak to interact with biological systems, and thus incapable of influencing physiological functions; biological functions and genetic material[1]. But, potential harmful ef-

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fects of power lines associated with residential rather than occupational exposure was the first report by[2] in 1979. By the early 1990s, numerous studies have been conducted on childhood cancer in various locations to confirm this result. Some of these studies confirmed that result[3-10], while others found no influence[11-14]. Cancer is caused by alterations in oncogenes, tumor-suppressor genes, and micro-RNA genes. These alterations are usually somatic events, although germ-line mutations can predispose a person to heritable or familial cancer[15]. Mutated versions of these oncogenes are found in many cancers, as a result of their over-expression. This leads to the unregulated expression of many genes, some of which are involved in cell proliferation and results in the formation of cancer. Recently, the effect of low frequency electromagnetic fields (LF-EMFs) on the expression level of the *c-myc* oncogene in human primary cell culture (PBMCs) was investigated by[16]. The author found that the expression level value of *c-myc* oncogene increased from 1.69% at 0.82mT to 4.65% at 4.67mT expression level value after the fourth day of exposure. The aim of this study is to investigate the effect of LF-EMFs (50 Hz) on the expression level of the *c-myc* oncogene in tumor processes in mouse mesenchymal stem cells (MSCs).

MATERIALS AND METHODS

Cell cultivation

MSCs. Mesenchymal stem cells (MSCs) were collected from healthy two mice (2-4 weeks old). Each mouse was killed by ether vapor and femur bones of the hind limbs were collected. The bones have been sterilized by 70% ethanol and then washed extensively with sterile 1X PBS. The two ends of each femur bone were cut and pre-warmed pre-filtered complete media Alfa MEM supplemented with FBS; Pen/Strep mixture and L-glutamine was forced through the bone using syringe passed through cell strainer 70 µm sizes. Single cell suspension of bone marrow tissue was prepared by pipette up and down. The cells were incubated till adhere and look nearly confluent cells appeared (after 8 days). Cells were washed twice with PBS to remove any excess serum. Trypsin/EDTA was added to the cells for 3-5 seconds. The action of Trypsin/EDTA was stopped by adding culture media supplemented with 20% fetal bovine serum (FBS). The detached cells were removed in 50 ml falcon tube and centrifuged at 1200 rpm for 5 minutes. Supernatant was discarded and cells pellet was transferred into a new cell culture flask. Cells were incubated in CO_2 incubator at 37ºC and ready for exposing to specific dose and time of LF- EMFs.

EL4-Lymphoma cells. EL4-Lymphoma cells were obtained from American Type Culture Collection (ATCC). Cells were frozen in cryo-vial using liquid nitrogen. Cryo-vials were removed from liquid nitrogen tank. Inside laminar flue cabinet frozen cells were transferred into 15 ml falcon tubes. Then, 10 ml pre warmed pre filtered RPMI 1640 culture media supplemented with 2 mM L-glutamine; 1M HEPES buffer and 20% fetal bovine serum (FBS) were added. Cells were centrifuged at 1200 rpm for 10 minutes to wash them and remove any excess freezing media. Supernatant was discarded and cells pellet was re-suspended in 75 cm^2 cell culture flask by Ham's F12 culture media. Cells were incubated in the CO_2 incubator at 37ºC. After 2 days, cells were examined under 2000X phase contrast microscope. Cells in the flask were washed twice with PBS and centrifuged at 1200 rpm for 5 minutes. Supernatant was discarded and cells pellet was transferred into a new 75 cm^2 cell culture flask. Finally, cells were ready for further analysis.

LF-EMFs exposure system design

Using exposure unit which performed specifically for this study (Figure 1), MSC's were exposed once for one hour to alternating current (AC; 50 Hz) electromagnetic flux density range: 0.38mT, 0.82mT, 1.25mT, 1.66mT, 2.13mT, 2.52mT, 2.79mT, 3.06mT, 3.71mT, 3.96mT, 4.08mT, 4.52mT (appliances's EMFs range). The simulation of LF-EMFs exposure was performed in an incubator designed for the cells exposure. The source of LF-EMFs was established by powerful wave generator which produces electromagnetic field in the range from 10 to 500 cps (cycle per second). The flux density of the field was measured by Hand-Held Gauss/Tesla Meter and controlled by adjusting the Volt (V) and Ampere (A) in the SF-9584 *low Voltage* AC/DC power supply.

Quantitative real time polymerase chain reaction (qRT–PCR)

RNAs extraction. In day 4 of exposure where the begging of the change in cells count and viability be-

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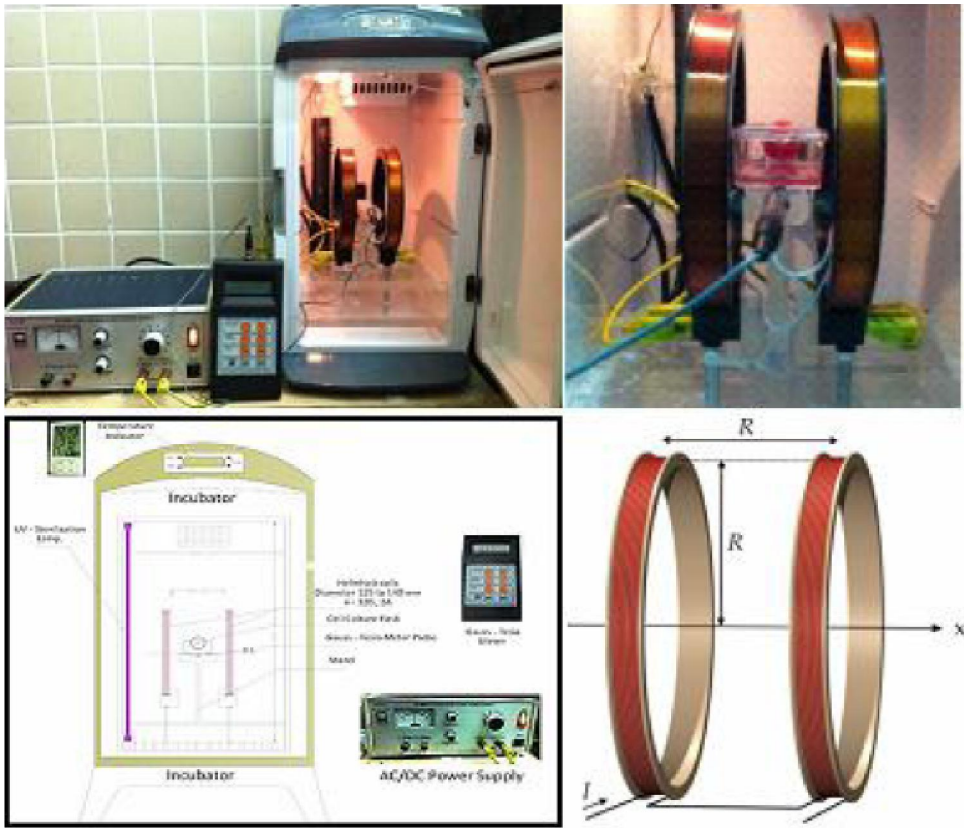

Figure 1 : Low frequency electromagnetic fields (LF-EMFs) exposure unit.

tween exposed and unexposed cells, total RNAs were collected from cells in day 4 and 14 after exposure in MSCs using Gene JET RNA Purification Kit (Thermo Scientific, USA). In order to make sure that RNAs isolation process were successful, total RNAs samples were separated on 1.1% agarose gel electrophoresis. The extracted RNAs from different samples were quantified and qualified using NanoDrop spectrophotometer. Total RNAs in samples were normalized with the same concentration by dilution to avoid any false increase in gene expression levels at 10 ng.

c-myc gene expression. Using SYBER Green 1-step qRT-PCR Kit (Thermo Scientific, USA), gene expression of *c-myc* (targeting gene) and *β-actin* (reference gene) was quantified by Real-Time PCR System (Thermo Scientific PikoReal) with the use of specific primers sequences (Forward/Reverse) 52 - TTCTCAGCCGCTGCCAAGCTGGTC-32 /52 - GGTTTGCTGTGGCCTCGGGATGGA-32 for *c-myc* mice gene and (52 - AGAGGGAAATCGTGCGTGAC-32 /52 - CAATAGTGATGACCTGGCCGT-32 for *β-actin* gene[17]. qRT-PCR was performed in a reaction volume of 10 µl using 0.1 µl verso enzyme mix, 5 µl 1-step QPCR SYBER mix (1X), 0.5 µl RT-enhancer, 0.7 µl forward and reverse primers (10 pm), 1-2.6 µl water (PCR grade) and 0.4-2 µl RNA template (10 ng). qRT-PCR program was applied as one cycle of cDNA synthesis at 50ºC for 15 minutes, one cycle of Thermo-start enzyme activation at 95ºC for 15 minutes and followed by 35 cycles of denaturation at 95ºC for 15 seconds, annealing at 65ºC for 40 seconds and extension at 72ºC for 1 minute.

RESULTS

Optimization of MSCs viability, morphology and count

MSCs were isolated and divided into unexposed cells (control) and exposed cells (treatment) with exposure range from 0.38mT to 4.52mT flux density for one hour exposure duration. Under 2000X phase contrast microscope, photomicrographs of MSCs were taken in days 4 and 14 after exposure showing differences in cells number and morphological characteristics due to different doses of LF-EMFs. In day 4, LF-EMFs exposure flux density ranged from 0.38mT to 1.66mT has no effective on cells number. While, LF-

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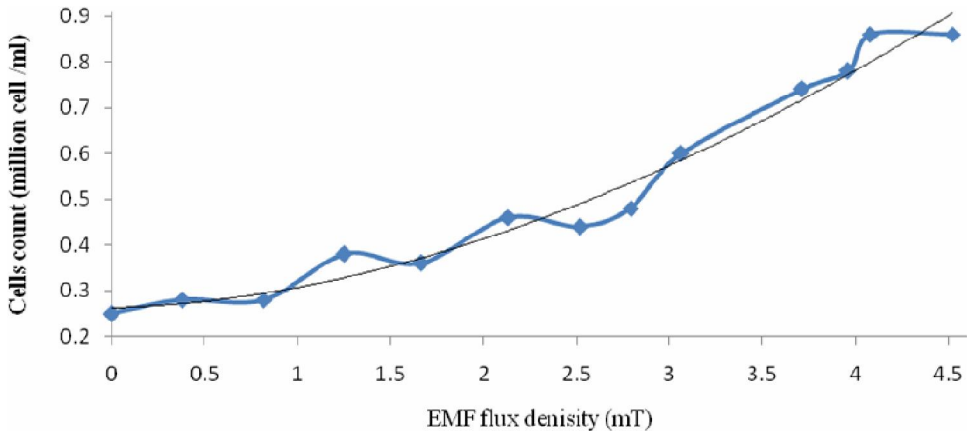

Figure 2 : Graph shows the relationship between EMFs flux density (mT) and cells count (million cells/ml) in day 4 after exposure.

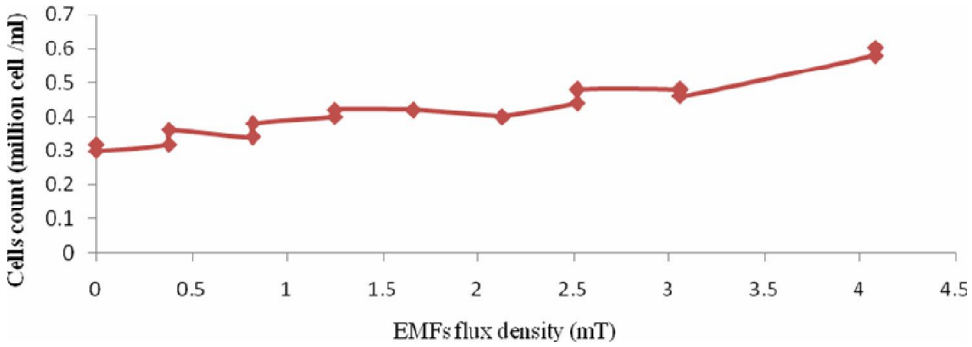

Figure 3 : Graph shows the relationship between EMFs flux density (mT) and cells count (million cells/ml) in day 14 after exposure.

EMFs exposure flux density ranged from 2.13mT to 4.52mT led to great increasing in cells number as a result of changes in cell cycle and mitotic division in exposed cells (Figure 2). In day 14, significant increasing in cells number caused by LF-EMFs exposure flux density ranged from 0.38mT to 4.52mT doses (Figure 3).

c-myc expression level

Due to the LF-EMFs exposure doses ranged from 0.38mT to 4.52mT, *c-myc* oncogene expression level changes in MSCs. In day 4 after LF-EMFs exposure, *c-myc* oncogene expression level increased from 3.61% at 0.38mT exposed cells reaching to maximum value 95.9% in 4.52mT exposed cells (Figure 4). Also, in day 14 after LF-EMFs exposure, *c-myc* gene expression level increased from 29.9% in 0.38mT exposed cells reaching to the maximum value 98.6% at 4.52mT exposed cells (Figure 5). When LF-EMFs flux density increases, *c-myc* expression level increases, affecting different cellular functions. A great increasing in *c-myc* oncogene (over expression) turning cells into cancer cells such as EL4-Lymphoma cancer cells which have 21% increasing in *c-myc* oncogene expression level.

DISCUSSION

In this study, the effect of LF-EMF (50 Hz) was investigated on the expression of the *c-myc* oncogene which is known to be involved in normal cell proliferation and possibly also in tumor processes in mouse mesenchymal stem cells (MSCs). The study, clearly illustrated that there is a significant effect of low frequency electromagnetic field (LF-EMF) on cells functions and *c-myc* oncogene expression levels. This effect can be different according to the exposure flux densities which were variable in this study within ranges 0.38mT to 4.52mT. However, MSCs cells count have significant increasing within range 2.13mT to 4.52mT in day 4 after exposures, which mean changes in cell cycle and mitotic division in exposed cells. LF-EMFs exposure doses more than 4.52mT decreased cells count of MSCs, indicating that cells respond to exposure by turning into necrotic cells and then die as a victim of extrinsic events beyond its control. In day 4 after LF-EMFs exposure, *c-myc* oncogene expression level increased from 3.61% at 0.38mT exposed cells reaching to the maximum value 95.9% at 4.52mT exposed cells. In

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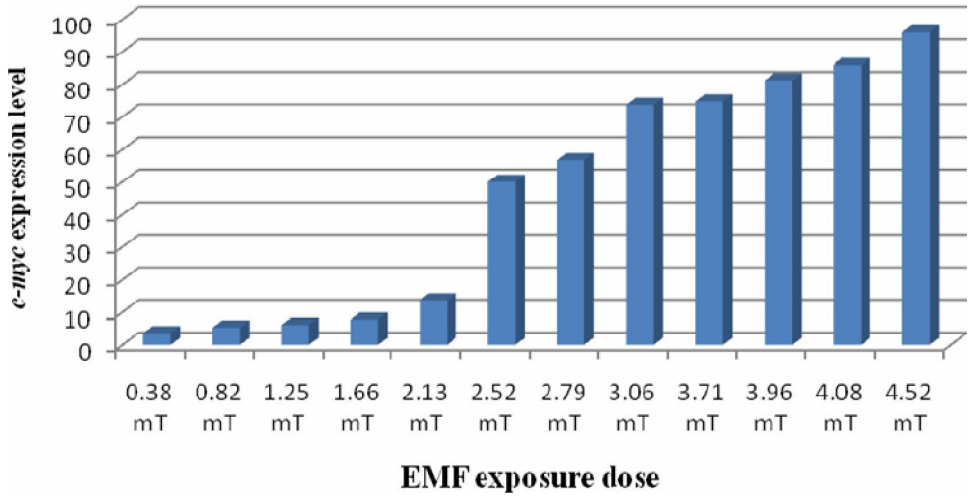

Figure 4 : The expression level of *c-myc* oncogene in exposed MSCs (%) compared with unexposed cells in day 4 after exposure to AC electromagnetic flux density range from 0.38mT to 4.52mT (milliTesla).

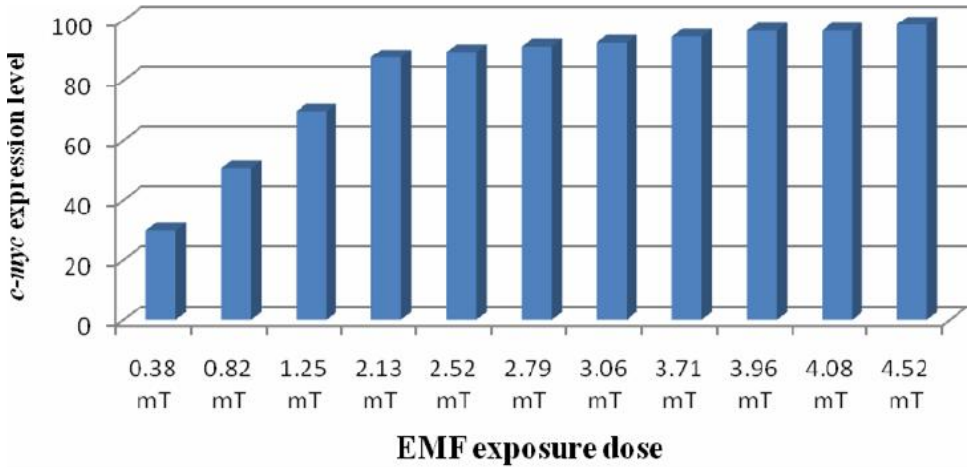

Figure 5 : The expression level of *c-myc* oncogene in exposed MSCs (%) compared with unexposed cells in day 14 after exposure to AC electromagnetic flux density range from 0.38mT to 4.52mT (milliTesla).

day 14 after LF-EMFs exposure, *c-myc* oncogene expression level increased from 29.9% at 0.38mT exposed cells reaching to the maximum value 98.6% (over expression) at 4.52mT exposed cells. Over expression of *c-myc* oncogene which generated by LF-EMFs causes a variety of human cancer diseases such as leukemia, nervous system tumors and lymphoma among children; and leukemia, nervous system tumors, and breast cancer among adults. We noticed that, when LF-EMFs flux density increase, *c-myc* oncogene expression level increases, great increasing (98.6% at 4.52mT, day 14 after exposure) turning cells into cancer cells such as EL4-Lymphoma cancer cells which have 21% increasing in *c-myc* oncogene expression level. The results in a previous study to the same author[16] and our current results join to the studies which confirmed the results of[2] and deserve attention because we are daily and continuously exposed to enormous amount of electromagnetic fields.

CONCLUSION

Human body is exposed daily to enormous amount of low frequency electromagnetic fields (LF-EMFs) in everywhere: outdoors, indoors and workplaces which can, in the long term, lead to alteration in human genetic materials causing different cancer diseases. The results in this study showed clearly that the increased exposure to LF-EMFs leads to increasing in expression level of *c-myc* oncogene in mouse mesenchymal stem cells (MSCs) reaching to the maximum value 95.9% at 4.52mT in 4 days only of exposure and 98.6% at 4.52mT in two weeks of exposure, causing damage in mammalian biological functions and genetic material. This study deserves attention because we are continuously exposed to increasing enormous amount of elec-

tromagnetic fields day after day.

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