Effect of ELF-EMF Exposure on Human Neuroblastoma Cell Line: a Proteomics Analysis

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Abstract

Background: Extremely low frequency electromagnetic fields (ELF-EMF) have been common in daily life all over the world. They have produced by power lines and electrical appliances, but higher levels of them have raised a lot of concerns about their carcinogenesis. Both epidemiological and laboratory studies have suggested that EMFs might increase cancer incidence, including acute childhood leukemia, brain and breast cancer.

Methods: In the present study, SH-SY5Y human neuroblastoma cell line has exposed to 2mT, 50 Hz magnetic field for 3 h. Next, effect of this exposure on protein expression including over-expression or under-expression has assessed by proteomics.

Results: Bioinformatics and statistical analysis using progenesis same spot software on the obtained 2D electrophoresis has shown that expression of 189 proteins in exposed group has changed relative to control. Besides, PCA analysis has verified results of clustering, and has shown that protein data has clustered according to experimental conditions.

Conclusion: The results of this study have shown that ELF-EMF changes cell morphology via altering protein expression, but more profound studies have needed to determine the kind of proteins altered.

Keywords: Electromagnetic fields; Proteomics; Neuroblastoma

Please cite this article as: Hasanzadeh H, Rezaie-Tavirani M, Seyyedi SS, Zali H, Heydari Keshel S, Jadidi M, Abedelahi A. Effect of ELF-EMF exposure on human neuroblastoma cell line: a proteomics analysis. Iran J Cancer Prev. 2014; 7(1):22-27.

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Received: 23 Jun. 2013
Accepted: 30 Aug. 2013
Iran J Cancer Prev. 2014; 1:22-27

Introduction

Environmental exposure to Extremely Low Frequency (ELF) Electromagnetic Fields (EMF) was commonly present in daily life all over the world and has increased considerably in parallel with growing technology. The potential carcinogenesis of ELF-EMF has extensively investigated [1, 2]. The leading studies on potential health hazards of environmental ELF-EMF exposure have performed in the former USSR in the 1960s [3, 4].

During the last few years various and profound investigations have been performed in laboratories world-wide to assess the biological effects of ELF electromagnetic fields [5]. Some epidemiological studies have reported positive evidence about existing correlation between exposure to ELF-EMF and increased incidence of certain forms of cancer especially acute childhood leukemia, lymphoma, brain and breast cancer particularly male breast cancer [6]. Moreover, there have also been a lot of in vivo & in vitro studies that if ELF-EMF could act as a promoter or co-promoter of cancer [7-10].

Some of these studies have not shown any ELF-EMF related biological adverse effects [1, 11] and scientists believe that it could be well accepted such fields have not possessed sufficient energy to generate direct DNA damage [3, 12, 13]. But in other studies, it has been demonstrated that ELF-EMF could affect on regulation, chromosomal structure, cell proliferation and apoptosis [14, 15].

Furthermore, the results of several in vitro studies have indicated that ELF-EMF was able to change the expression of proteins which have involved in control of cell proliferation [16].

In summary, biological effects of ELF-EMF have related to frequency, intensity, timing and duration of exposure and also intrinsic susceptibility of different cell lines [17]. Therefore, despite the large number of performed studies, the effect of ELF-EMF on cells has still remained unclear and controversial [18]. Conventional biochemical techniques could not easily found the biological effects of ELF-EMF on

neuronal cell biology. The proteomic approach was a powerful technique which was able to find subtle changes in protein expression during or after exposure to drugs, physical conditions, etc. [15].

In this study, due to uncertainty on the biological effects of ELF-EMF and its controversial results, the potential effects of 50Hz ELF-EMF with a magnetic flux density of 2 mT on the protein expression of SH-SY5Y human neuroblastoma cell line has studied using proteomics to find out the potential effects of these fields on human nervous system.

Materials and Methods

Cell culture

The SH-SY5Y human neuroblastoma cell line has obtained from National Cell Bank of Iran (NCBI, number: C611). This cell line has maintained in RPMI: Ham's F12 (1:1), 2 mM Glutamine, 1% Non-Essential Amino Acids (NEAA), 15% Fetal Bovine Serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C in an incubator containing 5% CO_2. All the cells have passaged twice weekly and used for experiments while exponentially growing. For experiments, cells have cultured in 75 cm^2 flasks set up at 3×10^5cells per flask in 10ml of RPMI.

Exposure system

EMF exposure system has produced homogenous ELF-EMF sinusoidal, 50Hz, 2mT has generated by a Helmholtz coil. This system has designed in two parts in which the inside the incubator without inducing any significant temperature rise inside the incubator. Human SH-SY5Y cells have exposed for 3h and then the effects of the ELF-EMF on morphology and proteome expression have analyzed.

Microscopic study

In order to find effects of ELF-EMF on cell line, cell morphology and pattern of cell distribution in the exposed and control cell has compared using inverted microscopy.

Protein Extraction

After 3h exposure to ELF-EMF, the harvested cells (exposed and control cell) have washed three times using washing buffer (250mM D-Sorbitol and 10mM Tris, pH=7.0). Subsequently, lysis buffer containing 8 M urea, 4% CHAPS (3-(3-cholamidopropyl) dimethylammonio-1-propanesulfonate), 40mM dithiothreitol (DTT), 2% pharmalyte (pH=3–10NL), 1mM Phenyl Methyl Sulfonyl Fluoride (PMSF) and 1mM ethylene diamine tetra-acetic acid (EDTA) have added. Each sample has been encountered to sonicate pulse for 5 min, and then centrifuged at 40000g for 30 min at 4°C.

Protein concentration has quantified by Bradford assay using BSA standards. The supernatants have extracted and stored at -20°C until using for electrophoresis. All steps of the protein extraction and proteomic analysis have performed in Proteomics Research Center (PRC) of Shahid Beheshti University of Medical Sciences.

Two dimensional SDS-PAGE

The first dimension electrophoresis has carried out with 17cm pH=3-10 IPG strips. To do so, 400µg of protein has loaded onto strips and three gels have run for each sample to reach reproducible results. Strips have rehydrated in a protocol lasting 4h without any voltage following by an 8 h part with 50 V.

The iso-electric focusing has programmed at a gradient mode, which has first focused for 3 h at the 500, 1000 and 8000 V, respectively, then has continued at 8000 V until a total of 50000 V/h (all steps with a maximum current of 50 µA for all strips. After the first dimension, strips have equilibrated for 20 min in the equilibration buffer containing 6 M urea, 30% glycerol, 2% SDS, 2% DTT, and then for 20 min in the same buffer except that DDT has replaced by 2.5% iodoacetamide.

In the second dimension, the treated strips have transferred onto 12% SDS-Polyacrylamide slab gel running in 2.5W each gel for 30 min and 15 W each gel until the bromophenol blue dye has reached the bottom of the gel. Gels have stained by Coomassie brilliant blue staining. Therefore, the gels have scanned by Bio Rad Image Scanner and 2-DE images have analyzed using nonlinear progenesis same spot software.

Results

Cell culture and microscopic study

Immediately after exposure, cell morphology has been studied using microscopic assessments (Figure 1). The morphological study has indicated that exposure of ELF-EMF (50 Hz, 2 mT) could affect viability and morphology of SH-SY5Y human neuroblastoma cell line.

Proteomic analysis

For besides, proteomics approach has used to determine differences in protein expression between exposed and control cells. In this technique, the proteins have separated first by their isoelectric point (pI) and then by their molecular weight. Figure 2 has shown the 2DE map of exposed and control cells. Comparison of protein patterns on these gels using progenesis same spot software has performed. Using this software, representative hierarchical cluster analysis (Figure 3) and principal components

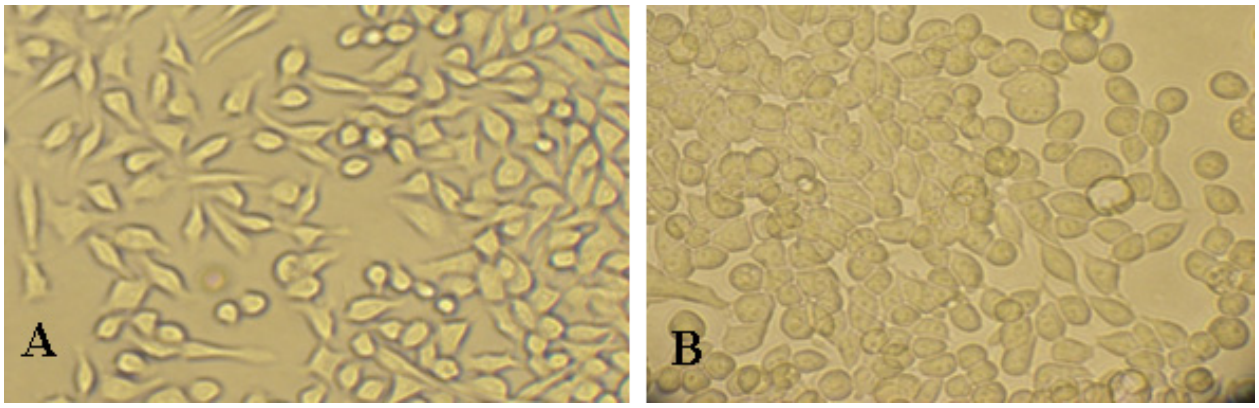

Figure 1. It has shown morphology of SH-SY5Y human neuroblastoma cell in the control (A) and exposed (B) condition.

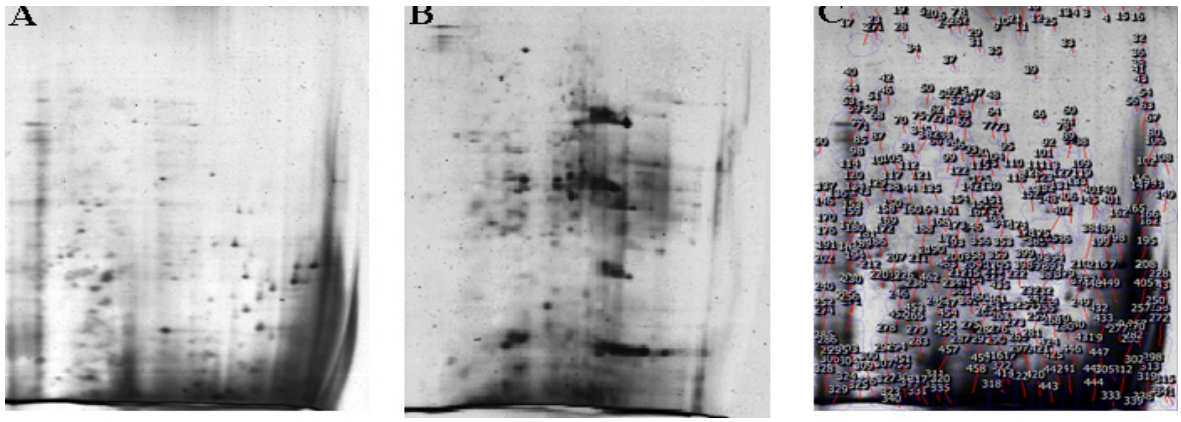

Figure 2. It has shown comparison of 2DE gels of control (A) and exposed (B) groups. The gel of normal cells has selected as reference gel and the spots have marked on it (C).

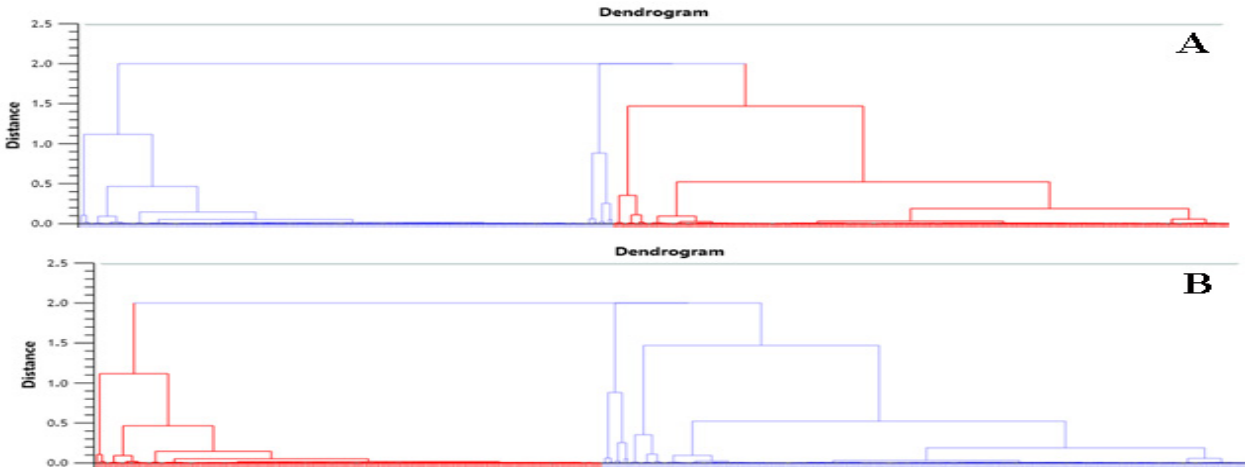

Figure 3. A: It has shown clustering proteins in exposed group with over-expression (red color). B: It has shown clustering proteins in exposed group with under-expression (red color).

analysis (Figure 4) of control and exposed gels have done. Our finding has shown that protein dendrogram of 189 proteins differentially has altered (*p-value*<*0.05*) in exposed cells. Some proteins have over-expressed and some of them were under-expressed. In this study, hierarchical cluster analysis and principle component analysis have demonstrated separation of the proteins, into both control and exposed groups (Figure 3). Figure 3A has shown the clustering proteins in exposed

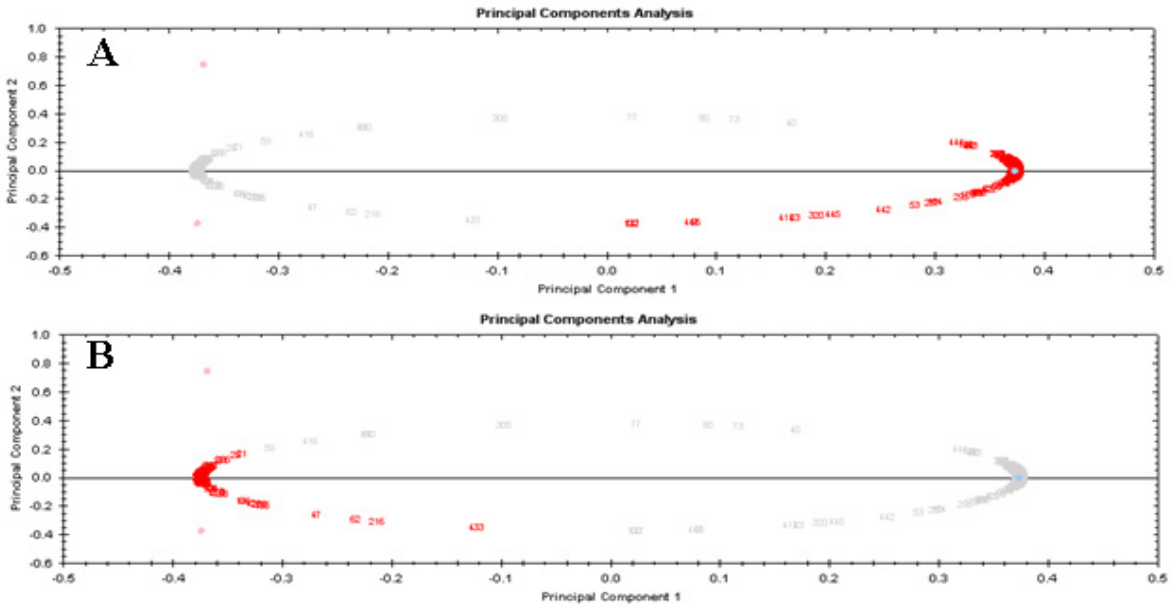

Figure 4. A: It has shown principal Components Analysis in exposed group with over-expression (red color). B: It has shown principal Components Analysis in exposed group with under-expression (red color).

group that have higher expression than control group and Figure 3B has shown the clustering proteins in control group, that had higher expression than exposed. In order to verify of clustering principal components analysis has applied (Figure 4).

Discussion

The results of the present study have revealed that exposure of SH-SY5Y human neuroblastoma cell line to ELF-EMF could alter cell morphology and protein expression. Gel analysis by progenesis same spot software has shown that some proteins have significantly over-expressed or under-expressed as a result of 50 Hz ELF-EMF exposure. Clustering was a logical method for proteins classification, showing schematic representation of their relation and also understanding of protein expression alteration [18, 19].

There have been a large number of reports to determine if the 50/60 Hz ELF-EMF generated by electrical appliances, which were mutagenic or genotoxic, and thus established a plausible mechanism for cancer risk [16-20]. However, despite the great number of studies that have been performed, the biological effects of ELF-EMF were still not clear and controversial. Proteomics was a robust technique to study protein expression process and rapidly change our approach to cancer research [19, 21, 22]. Therefore, it has seemed that proteomics and functional analysis had the potential to show if ELF-EMF promote cancer risk in cells or not [16, 22]. In spite of merits of this technique, there were a few studies which have assessed biological effects of ELF-EMF *in vitro* or *in vivo* using proteomics [16, 23]. Because of high susceptibility of neuronal cells to environmental stresses such as exposure to ELF-EMF, several studies have performed on gene expression in this cell line [15, 18, 24, 25], but we have found only one study about proteomic analysis of neuronal cell line. Sulpizio et al. in their work has exposed human SH-SY5Y neuroblastoma cells to a 50Hz, 1mT sinusoidal ELF-MF at 5, 10 and 15 days and has assessed proteome expression. They have shown that ELF-MFs exposure has altered the proliferative status and cytoskeleton organization, and then was able to trigger a shift toward a more invasive phenotype [26].

In conclusion, the findings of this study have indicated that ELF-EMF at above mentioned exposure conditions could affect cell morphology via alteration of proteins expression, but it has still needed more complementary studies to reveal more insight about the kind of protein that has altered ,and the intensity level of ELF-EMF which might be considered safe.

Conclusion

As discussed, the findings have referred to considerable alterations in protein expression of the radiated cells by ELF-EMF. So it could be concluded that more investigation about radiation patterns and also protection aspects of ELF-EMF have needed. It has seemed that more resolution about the effects of

ELF-EMF could lead to finding the new way for human health improvement.

Acknowledgment

This work was a part of a common project supported by Shahid Beheshti University of Medical Sciences and Semnan University of Medical Sciences.

Conflict of Interest

The authors have no conflict of interest in this study.

Authors' Contribution

Mostafa Rezaei-Tavirani, Hadi Hasanzadeh and Samaneh Sadat Seyyedi have designed the study, gathered and analyzed the data, and finally written the paper. Saeid Heydari, Hakimeh Zali and Majid Jadidi have contributed to study design, sample collection and indentation. Ali Abedelahi has helped the writing and overall correction of the manuscript.

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