



The 50 Hertz Extremely Low-Frequency Electromagnetic and the T-helper Balance

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Abstract

Objectives: Electromagnetic fields (EMFs) are developing in scientific areas while biologic and immunologic effects have been proven in many studies. The purpose of the present study was to determine the effect of 50 Hz EMFs in the manner of the whole body exposure on the T-helper balance (Th1/Th2) in rats.

Materials and Methods: This experimental research evaluated the impact of the EMF on T-helper balance, including 30 rats that were randomly divided into 3 groups. The control group and experimental groups were exposed to 50 Hz EMF with the intensity of 0.5 mT for one and two month(s), respectively. At the end of the exposure period, blood samples were collected from the left ventricle of the hearts of the rats and the serum levels of interleukin 4 (IL-4) and interferon gamma (INF- γ) were measured by the enzyme-linked immunosorbent assay and compared in all three groups.

Results: The level of IL-4 and INF- γ showed a notable change ($P=0.032$) during one month of EMF exposure, indicating a shift of T-helper balance toward the Th2 arm, meaning more strong allergic reactions and weaker immune responses against tumors and many other diseases. After 2 months, the levels of cytokines and the balance of the T-helper came close to the baseline (the control group samples), representing the adaptive trends of T-helper balance in longer exposures.

Conclusions: The results of the present research revealed that EMFs produced important changes in IL-4 and INF- γ levels and affected the T-helper balance shift toward the Th2 arm, implying stronger allergic reactions and weaker defense against tumor and various other diseases. However, understanding the true nature of these changes and their actual health effects requires further studies.

Keywords: Electromagnetic radiation, Immunity, IL-4, INF- γ

Introduction

Exposure to electromagnetic fields (EMFs) could impress the matter of health and disease. More precisely, modern life brings about EMFs with extremely higher intensities. EMFs are classified as potential carcinogens (1-4) and people are recommended to take precautions when using microwave ovens and living near high-voltage cables. The T-helper (Th) cells have important roles in mediating immune responses. There are two effector subsets of Th cells (Th1 and Th2). They produce specific cytokines that have different effects on the immune system. For instance, IL-4 produced by Th2 cells modulate the proliferation of Th1 cells and INF- γ produced by Th1 cells can alter the cytokine pattern of Th2 cells. Thus, these subsets have an antagonistic effect on each other's functions and will lead to opposite immune responses. Normally, the functions of these subsets are in balance, and the stimulation of any of them causes the inhibition of the other. Therefore, any factor disturbing this balance will be pathogenic (5, 6). Various studies have focused on factors affecting the immune system. The use of EMF as

a modulator of the immune system represents interesting clinical perspectives. Danger signals by EMF stimulate the immune response in addition to activating mechanisms that negatively regulate immune cell activation. Other studies have shown that EMFs would downregulate Th1 and upregulate Th17 responses (7, 8). The current research aimed to measure the effect of 50 Hz EMF on IL-4 and INF- γ patterns produced by Th cells in rats. The utilized method included exposures with shorter durations compared to the study by Salehi et al (7). Moreover, the frequency of 50 Hz is the operating frequency of most electric powers, and effects induced by this frequency could indicate more general influences compared to studies such as Ohtani et al (9). The effects resulting from the current investigation focus on the specific aspect of the immune system and the related outcomes.

Methods and Materials

Animals and Experiments

In this original research study, 30 Wistar rats (15 males and 15 females) with 8-week-old weighing 150 ± 10 g were

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Key Messages

- ▶ Exposure to the 50 Hz low-frequency EMF shifts the Th1/Th2 balance toward the Th2 cells.
- ▶ 50 Hz low-frequency EMFs caused significant elevated levels of IL-4 and reduced INF- γ levels.

separated from the animal house of the Tabriz University of Medical Sciences. All animals were kept under the standard situation and had access to free food and water. The temperature was maintained in the range of 22-25°C, and relative humidity was monitored at 40%-70%. The lighting was turned off or on under a 12-hour cycle. The animals were divided into 3 groups after 1 week of adaptation to the new condition. Each group contained 5 males and 5 females. Two groups were exposed to an EMF with a frequency of 50 Hz and a flux density of 0.5 mT for 2 hours per day. The EMF system equipment was based on the Helmholtz coil, which works following Fleming's right-hand rule. It constructed an alternate current of 50 Hz frequency. The EMF intensity could be controlled by a transformer. The EMF system equipment had 2 principal parts. In the first part, there were 2 copper coils placed one above the other separated by a distance of 50 cm between the coils (the exposure place). There was a tubular wooden vessel, the internal of which had a site for keeping the cages of the experimental rats. The second part was the transformer which checked the input and output voltage with a voltmeter and current with an ampere meter. The equipment was calibrated by the gaussmeter. A fan was fitted at the top and the temperature was checked to prevent an increase in temperature inside the chamber. The control group was kept in similar conditions with no EMF (sham exposure) because the external environment is effective in cytokine production. The first and second groups were underexposure for one month and two months, respectively, and then blood samples were obtained via a retro-orbital vein from all three groups. Finally, the serum levels of IL-4 and INF- γ were measured by sandwich enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instruction (10).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software for Windows, version 16 (SPSS Inc., Chicago,

IL) was used for statistical analyses following the per-protocol principle. All variables are presented as the mean and standard deviation (SD). Since the skewness and the kurtosis of all study variables were between -2 and +2, the distribution of the data was assumed to be normal. Levene's test was carried out to assess the homogeneity of variance between the study groups. The mean value among the groups was compared using the analysis of variances. Eventually, the Dunnett's test was used to identify the pairs with the control group. In this study, *P* values of <0.05 on the two-tailed test were considered statistically significant.

Results

In this comparative research, the results were compared in EMF exposure and the control group. Unfortunately, due to the death of some of the rats, 7 rats after one month and six rats after two months survived during the experiment. Our result showed higher levels of IL-4 while lower levels of INF- γ in the groups under EMF exposure in comparison to the controls. A significant increase in IL-4 levels whereas a significant reduction in INF- γ levels were observed after one month of exposure. Table 1 presents the *P* values of the differences regarding IL-4 and INF- γ among the EMF exposure and control groups. Based on the results, there is no significant difference between one-month exposure and two-month exposure groups for the two cytokines.

Discussion

The findings of the present study demonstrated that exposure to the 50 Hz extremely low-frequency EMF shifts the Th1/Th2 balance toward the Th2 arm, especially in the short term (one month). Nevertheless, adaptive alterations seem to occur in the long term (two months). As described earlier, Th1/Th2 cells are in balance with each other, and a shift toward the cytokine profile of the Th2 arm is associated with allergies and inefficient immune responses against tumors and many other diseases (5). This finding is in line with the fact that EMFs are classified as potential carcinogens (4) and are used as anti-inflammatory tools (3,11-13). The return of alterations toward the baseline in longer exposures (two months) indicates a homeostatic response to restore the balance. Such adaptive responses of immunity in chronic situations occur in many other exemplars, including the

Table 1. Differences of IL-4 and INF- γ Among the EMF Exposure and Control Groups

Cytokine	IL-4			INF- γ		
	Value	Pair	<i>P</i> Value	Value	Pair	<i>P</i> Value
One-month exposure	23.71 \pm 10.46	One-month vs. control	0.009	16.58 \pm 4.98	One-month vs. control	0.032 *
Two-month exposure	12.16 \pm 6.79	Two-month vs. control	0.037	24.25 \pm 20.08	Two-month vs. control	0.400 *
Control	10.2 \pm 8.12	One-month vs. two-month	0.627	33.4 \pm 20.72	One-month vs. two-month	0.402

Note. Values are presented as the mean \pm standard deviation. IL-4: Interleukin 4; INF- γ : Interferon gamma. *The significance level in comparison to the control group.

response to a reduction in Th (CD4+) cells in the acquired immune deficiency syndrome, where the count of Th cells increases in response to the present but hidden cellular immunodeficiency, which can last only for a while (14).

A similar study on the EMFs and the Th balance applied the exposure for three months and demonstrated that IL-4 and INF- γ had no significant alterations, and the balance of Th1/Th2 cells was similar to the control status. This is exactly in line with our conclusion about longer exposures, seemingly continuing with more compensation during the third month of exposure and vividly manifesting the reason for comparing one-month and two-month exposures with controls in the current study. Nonetheless, the true nature of these adaptive compensations is questionable and raises a question regarding whether the immunity in this state is in the normal condition or there is a hidden cellular immunodeficiency.

Many studies are investigating the effect of EMFs with different strengths and frequencies on biologic systems. These fields have relatively specific biological effects and are occasionally used in medical affairs (12, 15). EMFs have extensive biological effects. In 1979, it was found that the rate of death from cancer was higher in children living near the high-voltage lines of electricity, consequently, extreme attention was paid to the effects of electromagnetic carcinogenesis (4). In this regard, the findings of a review study revealed that "the findings are weak, inconsistent, and non-deterministic" (16). In the case of the reproductive system, another review study including epidemiological researches, ultimately reported that the relation between these fields and infertility requires more detailed evaluations (17). Mice exposed to the 50 Hz EMF experience changes in the testis tissue, leading to infertility (18). Based on the results of another study, the effect of EMFs with the frequency of 4 MHz on mice was destructive and had fundamental physiological changes in brain activity, cellular communications, and humoral immunity (19). In the case of high-frequency fields, a wide variety of changes resulted from the distribution of body hemostasis such as variations in nervous, endocrine, and immune systems (15). A previous study demonstrated that most biological effects are related to the immune system and EMFs can affect them. For example, melatonin had an important role in the immune system, and EMFs affected the reduction of this hormone (20). The results of similar research indicated that the effects of EMFs with a 60 Hz frequency rate on immune and endocrine systems are linked together (21). Another investigation on 60 Hz fields examined the related effects on 20 different immune parameters and concluded that changes in parameters were made in nonlinear patterns, and more precise methods should be used to evaluate these changes in order not to deny the confounding influences (22). Other studies on women living near the broadcast station showed that the high-frequency EMF (500 kHz-3 GHz) reduces cytotoxic activity in the bloodstream and

this effect is dose-independent (2,23). According to these reports, it is likely that the features of the EMF effects could be non-linear or dose-independent depending on the applied EMF. Inconsistencies exist in these studies about EMFs and immunity. On the other hand, some studies reported no significant alterations of some parameters after applying EMFs both in high and low frequencies (7,24). Moreover, contrary to what was mentioned before, other studies demonstrated that EMF has a stimulatory effect on immunity. For example, applying multiple high-frequency fields increased immunity (15). Fields with a frequency of 50 Hz, which is a frequency similar to the present study, increase phagocytosis. This increase in phagocytosis is due to the release of free radicals which are carcinogenic agents (25-27).

Several other studies reported the anti-inflammatory effects of high-frequency fields (11, 12, 28), as well as the apoptosis of T-lymphocytes (11, 29), which can be beneficial in some medical situations. It was found that high-frequency fields induce a reduction in natural killer cell (NKC) and INF- γ levels in the peripheral blood (30). Many studies pointed to the effects of EMF therapy on the immune system. Most of these therapeutic effects have been beneficial in reducing inflammation and improving tissue repair. Therefore, such non-invasive therapies have the potential of being "alternative and complementary medicine" (8,31,32). A better understanding of the mechanisms of these therapeutic devices will help optimize them. As described above, extremely high and low frequencies are commonly used for these applications. Several studies evaluated the effects of EMF on cytokines in mice. These studies have considered TNF alone or in combination with other cytokines, used fields with high frequency and low intensity, and showed that the production of TNF- α , IL-4, and some other cytokines increased while the production of IL-1B and IL-6 represented a reduction. Changes in the frequency or intensity of EMF or the maintenance of experimental animals can affect these changes (33, 34). As mentioned earlier, studies about various EMFs affecting cytokines, without regarding the Th balance, report reductions in INF- γ (30) and elevated IL-4 (35, 36), which is consistent with the results of the present investigation. Therefore, the obtained results seem to occur with EMFs with other frequencies and intensities as well. It is of note that the modern world exposes us to various EMFs that have disruptive influences on health and biologic systems although they bring about emerging noninvasive therapeutic potentials.

Conclusions

EMFs caused significant changes in IL-4 and INF- γ within one month in this study, causing the Th balance shift toward the Th2 arm, meaning stronger allergic reactions while weaker defense against tumors and many other diseases. After two months, cytokines and the Th

balance came closer to the baseline of the control group. Notwithstanding, understanding the true nature of these alterations and their actual health outcomes necessitates further investigations.

Authors' Contribution

BHK and BR: concept and design. MZ, AAK and MA: data collection and interpretation of the data. PL and ASL: performing of the study and writing of the draft. All authors read and approved the study.

Conflict of Interests

None declared.

Ethical Issues

The research protocol was approved by the Tabriz University of Medical Sciences, Tabriz, Iran.

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