

THE EFFECT OF HIGH FREQUENCY (27.11 and 2450 MHz) ELECTROMAGNETIC FIELDS ON IMMUNITY MODULATION IN MICE

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ABSTRACT

The classical problem in studies concerning the biological effects of high-frequency electromagnetic fields is the establishment of the relationship between physical characteristics of the fields and the magnitude of the effect. The first step in solving the problem is by the quantification of the relationship between properties of the exposure fields and the absorbed energy. Chronic stressor for individual may induce disorder of stress cell by high frequency electromagnetic field. The pathogenesis of the disorder is poorly understood. Lymphocyte cell has two types of immunity, i.e. innate and adaptive immunity. The high-frequency fields were applied to a transversal electromagnetic cell designed to allow microscopic observation of the lymphocytes and plasma cell during the presence of the high-frequency fields. This experimental research using separate sample pretest-posttest control group design was intended to explain such mechanism. This research used pathobiology paradigm with stress immunocompetent cell concept, and multivariate analysis design. With random assignment, a number of 84 *Mus musculus* Balb/C were divided into 12 groups (pretest 3 groups, control 3 groups, posttest P1 3 groups and posttest P2 3 groups). Samples were male mice of 3 - 4 months old, with the body weight of 28 - 32 grams. High frequency electromagnetic fields as stressor (with the frequencies of 27.11 and 2450 MHz, electromagnetic field intensity of 10 mT, 18 V/m) was exposed for 20, 25, and 30 days. The dependent variables were IFN-gamma, IL-10 producing lymphocytes and IgM, as well as IgG producing plasma cells. Result showed that stress lymphocytes and plasma cells, due to high frequency electromagnetic fields (27.11 and 2450 MHz) for 20, 25, and 30 days, decreased IFN-gamma-producing lymphocyte, IgG-producing plasma cell, and increased IL-10 producing lymphocyte and IgM-producing plasma cells ($p = 0.000$). Based on discriminant model, it was observed that stress immunity modulation due to high frequency electromagnetic field for the frequency 27.11 and 2450 MHz showed no remarkable difference. In conclusion, at high frequency electromagnetic fields (27.11 and 2450 MHz), the adaptation of immunity modulation occurs and earlier fatigue of lymphocytes becomes potential.

Keywords: high frequency electromagnetic fields, pathobiology, stress cell, immunity

INTRODUCTION

The exposure of environmental high frequency electromagnetic field is increasing along with the advanced use of electrical instruments, particularly in medical and industrial setting. High frequency electromagnetic field, also designated as microwave, is an electromagnetic wave radiation at the frequency of 3 MHz - 300 GHz. Its increasing use requires the understanding of the basic mechanism of therapeutic and possible adverse effects to disclose the influence of high frequency electromagnetic field in stimulating lymphocyte. The high frequency electromagnetic field has a potential to penetrate various types of barriers including biological materials, such as the body of human beings and animals (Schwan, 1980). This study investigated the presence of difference in IL-10 and IFN-gamma cytokines-producing lymphocyte and IgM,

IgG antibodies-producing plasma cells in group with stress cell exposed to electromagnetic field in the dose of 1 - 6. This study was aimed to disclose the effect of electromagnetic field exposure at the frequency of 27.11 and 2450 MHz on immunity modulation in mice (*Mus musculus* Balb/C), and to prove the difference in IL-10, IFN-gamma-producing lymphocyte and IgG, IgM-producing plasma cells in groups exposed to electromagnetic field at the dose 1 - 3 (magnetic field intensity 10 mT, electrical field 18 V/m, length of exposure 30 min/day for 20, 25 and 30 days) and control.

METHODS

This was an experimental study using male mice as experimental animals. These animals were exposed in vivo to high frequency electromagnetic field (27.11 and 2450 MHz). This study used separate sample pretest-posttest control group design. Samples comprised 84 homogeneous male mice aged 3 - 4 months with bodyweight of 28 - 32 grams. Samples were allocated in random into 12 groups, comprising pretest 3 groups, control 3 groups, posttest P1 3 groups and posttest P2 3 groups.

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The independent variable was electromagnetic field stressor produced from high frequency (27.11 and 2450 MHz) Diathermy exposed to the cage. Dose was varied in length, power, and frequency. The dependent variables were the cytokine IL-10 and antibodies IgM and IgG, which were morphofunctionally examined using immunohistochemistry. Control group comprised male Balb/C strain mice, aged 3 - 4 months, with bodyweight of 28 - 32 grams, kept in the cage at Electromedic Laboratory, 30 cm from the sample, and given with pellet and water. This study was carried out at the Department of Anatomic Pathology, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, and Department of Clinical Engineering, Health Polytechnic, Surabaya.

RESULTS

Validity test was undertaken to find the consistence of observation carried out by the author. Data obtained by the author were compared with those by the second observer. The results were subsequently subjected to statistical tests, either Manova or Anova. Homogeneous test with the variables of IFN-gamma, IL-10, IgM and IgG for pretest, control, and posttest P1 was carried out for the length of treatment days. This test was to prove that the length of treatment days was homogeneous. The results showed that the length of treatment days in pretest group had a Wilks Lambda value of 0.551 and $p = 0.280$, in control group, Wilks Lambda value of 0.577 and $p = 0.340$, and in Posttest P1, Wilks Lambda value of 0.484 and $p = 0.156$. Immunity modulation discriminant test was carried out to control and posttest P1 groups. This test was performed to prove the change resulting from the exposure to high frequency electromagnetic fields of 27.11 MHz and 2450 MHz.

Table 1. Results of immunity modulation discriminant test for control, posttest P1, and P2 groups

	Control		Posttest P1		Posttest P2	
	Mean	SD	Mean	SD	Mean	SD
D_IFN- γ	0.38	1.77	0.0476	0.86	0.19	1.57
D_IL-10	0.52	2.23	2.1	2.39	3.38	2.65
D_IgM	0.0476	0.50	0.9	2.49	-1.00	1.45
D_IgG	0.67	8.18	-5.9	6.62	-8.00	6,06

Wilks Lambda 0.519, $p = 0.00$

Discriminant analysis was performed to determine the discriminant variable of immune response endurance from posttest P1 and posttest P2 groups. In this analysis, variable function was simplified based on physiological

conditions that had different effects on immunity modulation, particularly in the formation of immunoglobulin and cytokines as shown in Table 1.

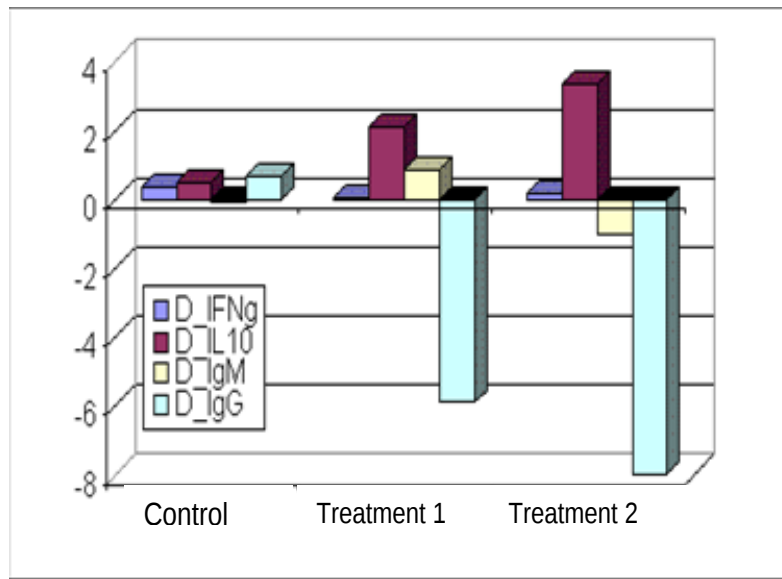


Figure 1. The average of immunity modulation

The pattern of immune response modulation in lymph nodes during stress was created from the results of Manova test, discriminant analysis, and contribution values (Fisher's linear coefficient) of the interacting discriminant variables. The contribution of each discriminant variable was obtained from the average of certain variable and other variables, which were controlled, and entered into discriminant function, and finally resulting in discriminator scores. The function

contribution of each discriminant scores (Table 2), presented as bar diagram, was regarded as the pattern of modulation. Table 2 shows difference in the discriminatory function contribution between control, posttest P1 and P2 groups. It can be seen that posttest P1 group has an increased IgM and IgG contribution, while that from IL-10 has decreased contribution. In posttest P2 group, all three variables demonstrate increasing contribution.

Table 2. The immunity contribution pattern between control and posttest P1 and P2 groups.

Variables	Control	Posttest P1	Posttest P2
IL-10	0.0458	-0.06652	0.5095
IgM	-0.0185	0.5139	0.168
IgG	0.3993	0.5751	1.288

Wilks Lambda 0.762, $p = 0.013$

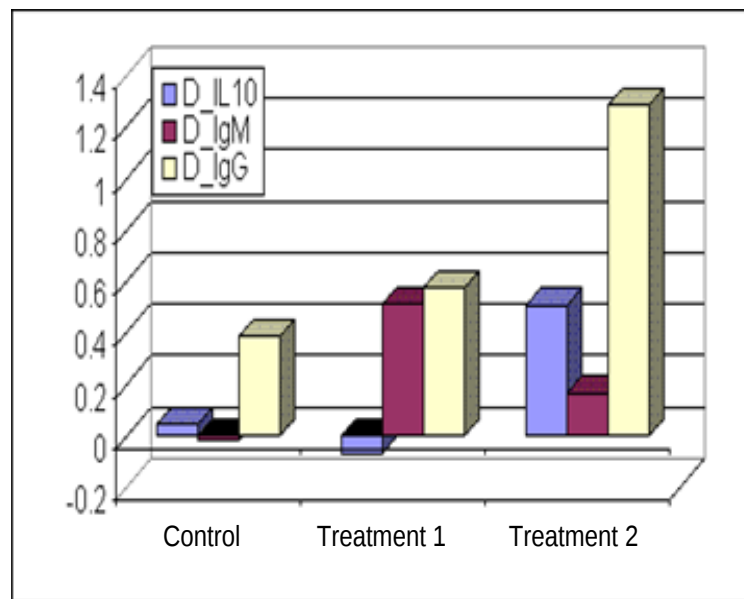


Figure 2. The immunity contribution pattern between control and posstest P1 and P2 groups.

DISCUSSION

This study used multivariate statistical analysis, since immune modulation is defined as biological change of immune system resulting from the interaction of all related variables. These variables were derived from research conceptual framework that was based on pathobiological paradigm using stress cell (lymphocyte) concept. Conceptual observation revealed that immunity modulation involved multiple variables. The use of multivariate test could prove and elaborate the mechanism of immunity modulation due to high frequency electromagnetic field stressor for 20, 25, and 30 days with magnetic field intensity of 10 mT and electrical field of 18 V/m. If the results of the test showed difference, this difference of immunity modulation could have resulted from the interaction with stressor.

Discriminant analysis was also carried out in this study to obtain predominant discriminant variables involved in immunity modulation in pretest, control, and posttest P1 groups with different stressor dose given. This discriminator was needed to create discriminant pattern reflecting the contribution of each discriminator function in the process of immunity modulation. The discriminant pattern was created from discriminant scores, resulting from the multiplication of Fisher's linear coefficient with each discriminator, with the assumption that other discriminators were equal to 0.

Each discriminator had its own magnitude of function contribution for each group. Therefore, if there were two groups, each discriminator would have had two Fisher's coefficients (Sharma, 1996).

The creation of discriminator pattern as the conceptual pattern was intended to support in explaining immunity modulation mechanism resulted from high electromagnetic field stressor (27,11 MHz and 2450 MHz) for 20, 25, and 30 days. This was a complementary model of the magnitude of discriminator function contribution in generating immunity modulation. The discriminant pattern is described into a bar diagram, intended to simplify the contributory pattern of each discriminator in the process of immunity modulation. The variables in this study were cytokine cell IFN-gamma and the antibodies IgM and IgG, which were examined morphofunctionally using immunohistochemistry. Morphofunctional examination of both cells still, however, contained subjectivity. To ensure the results of examination, the author was assisted by a second observer, a researcher who was experienced in observing the cells since 1984.

The observation to the morphofunctional appearance was not aimed to establish diagnosis. Instead, it was intended to identify variables, i.e., cytokine-producing lymphocyte and immunoglobulin-producing plasma cell. For this purpose, we used monoclonal antibody specific to the intended cytokine and antibody so that

they could be observed in examination using light microscope. For the examination of IFN-gamma-producing lymphocyte, we used the IFN-gamma monoclonal antibody, and IL-10 monoclonal antibody, was also used to examine IL-10-producing lymphocyte. Similarly, immunoglobulin-producing plasma cells were examined using monoclonal antibody against immunoglobulin. Cell counting was carried out full-sectionally by means of graticulae that dividing the visual field of light microscope in certain size in order to facilitate reading and prevent cell count duplication. Data were obtained from the average count in five visual fields in the graticulae. The observation was undertaken by the author and the observer to prevent subjectivity. To ascertain that resulted data had identical variation, independence from previous data, and had a normal distribution, we used ACF (Autocorrelation Factor) and Normal Probability Plot.

The difference of immunity and immunity modulation in lymphocytes, both in control as well as in posttest P1 and P2 groups, should be elaborated. This required further analysis, i.e., the discriminant analysis and the creation of discriminant pattern. Discriminant analysis was used to obtain discriminator and the magnitude of discriminator contribution to the biological process of immunity modulation. The discriminator variable is expected to become a predominant factor in biological response, while the magnitude of contribution served to indicate how much a variable contribute to the biological response. The magnitude of discriminator contribution was used to analyze immunity difference in lymphocytes exposed to high frequency electromagnetic field (27.11 MHz and 2450 MHz). Table 1 on the results of manova test shows modulation difference in control, posttest P1 and P2 groups ($p = 0.00$), while anova test revealed that all variables were different, except IFN-gamma.

Furthermore, discriminant analysis was undertaken to four variables, from which three discriminators were obtained. In descending order according to their discriminating power, they were IgG, IgM, and IL-10, which were classified as true for group originality (61.9), and true for cross validity (58.7%). It should be noted that the magnitude of contribution was not reflecting average data of each variable. It was the result of multiplication of Fisher's coefficient with the average of each discriminatory variable that described the contribution of immunity modulation discriminator. Furthermore, the composition of contribution magnitude was mapped in a pattern and, referring to the conceptual framework, this pattern could explain the mechanism of immunity modulation resulting from the exposure of high frequency electromagnetic field as stressor to the lymphocytes.

Modulatory pattern as seen in the bar diagram (Figure 2) shows three different patterns, the discriminatory pattern of control, pretest P1 and posttest P2 groups. The three patterns were used to elaborate the conceptual framework of this study as an immunopathobiological explanation of immunity modulation of T and B lymphocytes exposed to high frequency electromagnetic field (27.11 MHz and 2450 MHz) as stressor.

The modulatory pattern of posttest P1 group showed that the contribution of IL-10 lead to negative direction (-0.06652), IgM and IgG to positive direction (0.5139 and 0.5751, respectively). In posttest P2 group, the contribution of IL-10 lead to positive direction (0.5095), IgM to positive direction (0.168), and IgG also to positive direction (1.288). In general, these changes showed that the effect of stressor, presenting as electromagnetic field with frequency of 27.11 MHz and 2450 MHz for 30 days, increased Th2 lymphocyte, indicated by a high increase in the production of IL-10-producing cytokine. This resulted in the increase of IgM production and the suppression of IgG production. This proved that Th1 and Th2 cells were at the adaptation stage.

CONCLUSIONS

1. The higher electromagnetic field does not affect the modulation pattern of IFN-gamma, while IL-10 and IgG are increased and the IgM is reduced. The change of modulation pattern remains at adaptation stage, which may lead to exhaustive stage.
2. The immunopathobiogenesis of immunity modulation in stress cells should be further investigated to establish pathobiological studies using stress cells concept.

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