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Research Article

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# Effect of 6 GHz Radiofrequency Electromagnetic Field on Cytokine Levels in Rats

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## Abstract

***Purpose:*** *While technological devices are continuously developing at an incredible speed, the exposure to radiofrequency electromagnetic fields (RF-EMF) created by these devices is gradually increasing. In this study investigated the effects of 6 GHz (0.083 W/kg SAR) RF-EMF on rat cytokine levels.*

***Materials and Methods:*** *In this study, 20 Wistar Albino adult male rats ranging in weight between 250-300 g were used. Rats were divided into two groups as; Control and radiofrequency radiation (RFR) group. The control were not subjected to any treatment. RFR group rats were placed in a special treatment cage and exposed to 6 GHz RF-EMF for 4 hours/day for 6 weeks. After RF-EMF application, blood (2cc) were collected from all rats by cardiac puncture method. The plasmas of the collected blood were separated from the blood samples within the same day. Pro-inflammatory cytokine (TNF-α, IFN-γ) and anti-inflammatory cytokine (IL-4, IL-10) levels were analyzed as spectrophotometrically by ELISA method.*

***Results:*** *We found a statistically significant increase in plasma levels of TNF-α and IL-4 rats in the RFR group compared to the control ($p<0.05$). We also found an increase in the plasma level IFN-γ and IL-10, but they have no statistically significance ($p>0.05$).*

***Conclusion:*** *6 GHz RF-EMF exposure had significant effects on rat cytokine levels. It supported both pro-inflammatory and anti-inflammatory cytokine release.*

**Keywords:** 6 GHz Radiofrequency-Electromagnetic field, Cytokine, Plasma, Rat

## 1. Introduction

In parallel with the incredible speed and continuous development of technological products, these devices that we use in daily life make our lives easier, while also causing some disadvantages. The exposure to RF-EMF emitted from these devices, which are constantly developing, is increasing more and more. EMF sources are located in the frequency range of the electromagnetic spectrum (3 kHz - 300 GHz) and are widely used in daily life; in many areas including mobile phones, radio and television broadcasts, wireless communication networks, base stations, radio frequency identification systems, industrial and medical applications can be cited as examples [1]. The occurrence of some complications that occur as a result of RF-EMF interaction mechanisms with the body over long periods of time and their possible negative effects cause an increase in public health concerns [2,3].

Until today, numerous surveys, in-vivo, in-vitro and epidemiological studies have been conducted using human and animal modeling on the health effects of RF-EMF exposure [4-6]. The energy communication, distribution, penetration, absorption, and power storage of the exposed RF-EMFs with biological tissues are determined by the electric and magnetic fields generated by the body [7]. These parameters may differ from individual to individual depending on body size, age, gender, and physical characteristics. As a result of the disruption of the natural EMF

balance in the human body, structural and functional differences may occur in the cells [8].

The effect of acute or chronic exposure limits to RF-EMF on immune system cells and other systems has not yet been fully determined. Recently, studies in which the effect of RF-EMFs with different frequencies on cytokines has been investigated using human and experimental animal models have focused more on cytokine activity [9,10]. However, it is seen that the data obtained in the results of the studies conducted in this direction are not fully compatible with each other. Different experimental conditions or individual differences can be cited as the reason for the occurrence of this condition.

Cytokines can be defined as soluble mediators that carry signals between cells. These are multi-functional polypeptides that can be synthesized by various cells in the body (monocytes, lymphocytes, macrophages, and some somatic cells), similar to hormones, but with a low molecular weight (20-30 kDa). Cytokines are a group of regulatory proteins that are effective in the physiopathology of diseases, have therapeutic potential, and play a central role in the immune system by regulating immune responses [11]. Cytokines have mechanisms to regulate the inflammatory response by transmitting pro-inflammatory and anti-inflammatory signals [12]. Cytokines have various biological effects on the immune response to inflammation and infection stimuli. These may be listed as activation or inhibition of cell proliferation, cytotoxicity and/or apoptosis, cell growth and differentiation, chemotaxis, homeostasis, antiviral activity, inflammatory response, regulation of membrane surface protein expression, and elimination of pathogens [13-15].

There is an international competition for the development of RF-EMF systems. The studies conducted on the effect of acute or chronic exposure caused by these new systems on cytokine levels are quite limited. In particular, immune system cells are known to adapt to various perturbations caused by environmental stimuli. However, there are hardly any satisfactory studies reporting the value/values of RF-EMFs with varying frequency ranges that can be tolerated or adapted by the immune system. Considering this situation, in our study, we aimed to investigate the effects of long-term (42 days) 6 GHz (0.083 W/kg SAR) RF-EMF exposure on pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokine levels at rat plasma levels. The frequency of technological devices used in daily life is gradually increasing [16]. Therefore, we used 6 GHz RF-EMF in our study.

## 2. Materials and Methods

### 2.1. Supply of Experimental Animals and Equipment Design

This study was conducted in accordance with Decision No: 4/11 taken by the Local Ethics Committee of Experimental Animals of Çukurova University. The study was funded by Cukurova University Scientific Research Projects Coordination Unit (TDK-2021-13488). In the study, 20 Wistar Albino adult male rats ranging in weight between 250-300 g, bred at the Cukurova University Health Sciences Experimental Application and Research Center (SABIDAM), were used. During the experimental period, all rats were housed in a laboratory environment with an ambient temperature of 24±20C and humidity of 40-60% in accordance with the circadian rhythm (12 hours of light/dark) and fed with adlibitum pellet feed and tap water.

In the study, two Faraday cages with a size of 100x100x90 cm were used in order to protect the experimental animals from the possible negative effects of RF-EMF sources and to limit the effect of electromagnetic radiation created in the experimental setup. In our study, Faraday cages were used for both control and experimental groups. Before any application to the RFR group rats, the rats were removed from their standard cages and placed in a special application cage (consisting of 4 equal sections, 90x90x42 cm) in the Faraday cage, and adaptation to the environment (4 days) was achieved.

### 2.2. RF-EMF Application

In the study, Set Electronic, Co. for RF-EMF application. Ltd. (model:FR6GX-2W, Sakarya, Turkey) designed by; power supply 220~230 V, operating temperature -40°C~+60°C, fixed frequency modulation and frequency 5.8 – 6 GHz, power 0-2 Watt (adjustable), continuous wave (CW) sinusoidal RF generator with 50 Ω output was used. A monopole antenna with a quarter wavelength (λ/4) consisting of a single conductive plate and evenly distributed in all directions, coming out of the RF generator, was used. Before RF-EMF application to rats, the monopole antenna coming out of the RF generator placed at the upper midpoint of the Faraday cage was fixed in the middle of the special application cage (ground height: 35 cm) in such a way that it spreads evenly at the same distance to the subjects body.

### 2.3. Specific Absorption Rate (SAR)

Before the experiment, while the RF generator was turned off and the power was running at 1 Watt, the reflection and exposure levels from the four corners of the cage were normalized by measured with the ElectroSmog meter (TES-593, Taiwan) device in the area close to the monopole antenna (the middle point of the special application case). The electric field (EF) of RF-EMF was recorded as 6.29 ± 4% V/m, the magnetic field (MF) was recorded as 0.016 ± 9% A/m and the power density (PD) change was recorded as 11.80 μW/cm$^2$. These measured values are based on the EF, MF and PD values calculated in previous literature for the same conditions, orientation and antenna power [17,18].

The Sim4Life 7.0.2 program based on the Finite-Difference Time Domain method (FDTD; Finite-Difference Time-Domain) belonging to Zurich MedTech AG (Zurich, Switzerland) was used to calculate the SAR values. Three-dimensional model simulations of a volumetric pixel (voxel) rat were created using the ViZoo 1.0 (Virtual Zoo) program owned by Zurich MedTech AG. The IEE/IEC62704-1 averaging method was used for the simulation. Spatial-average SAR distribution was performed for 1 g average mass in the voxel rat model. In our study, the SAR value was calculated as 0.083 W/kg on the whole body in adult rats (Figure 1).

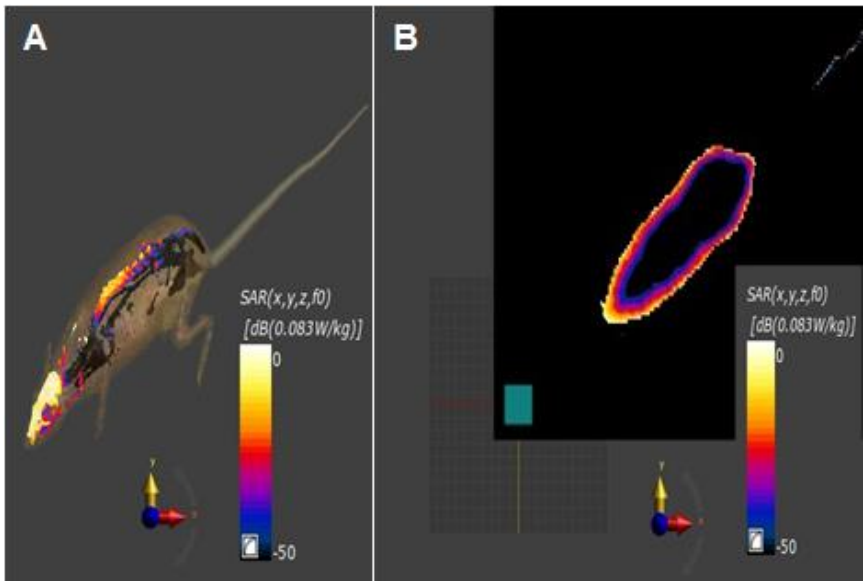


**Figure 1:** SAR simulations at 6 GHz. A) Adult rat voxel model, B) adult rat horizontal cross-section whole body SAR distribution for 1g average mass.

### 2.4. Establishment of Experimental Groups

In order to determine the effect of 6 GHz (0.083 W/kg SAR) RF-EMF on pro-inflammatory cytokine (TNF-α and IFN-γ) and anti-inflammatory cytokine (IL-4 and IL-10) levels in rat blood plasma levels by Enzyme-Linked İmmunosolbent Assay Method (ELISA), the study was divided into 2 groups: Control and RFR group.

**2.4.1. Control Group:** In this group, a total of 10 adult male rats were used. The rats were placed in one of the Faraday cages together with the standard cages in which they lived. Rats in this group were protected from possible electromagnetic radiation in the external environment and were not exposed to any treatment (Figure 2B).

**2.4.2. RFR Group:** In this group, a total of 10 male rats were used. The rats were placed in a Faraday cage and after a 4-day adaptation period, they were exposed to 6 GHz (0.083 W/kg SAR) RF-EMF for 4 hours/day for 6 weeks (Figure 2A).

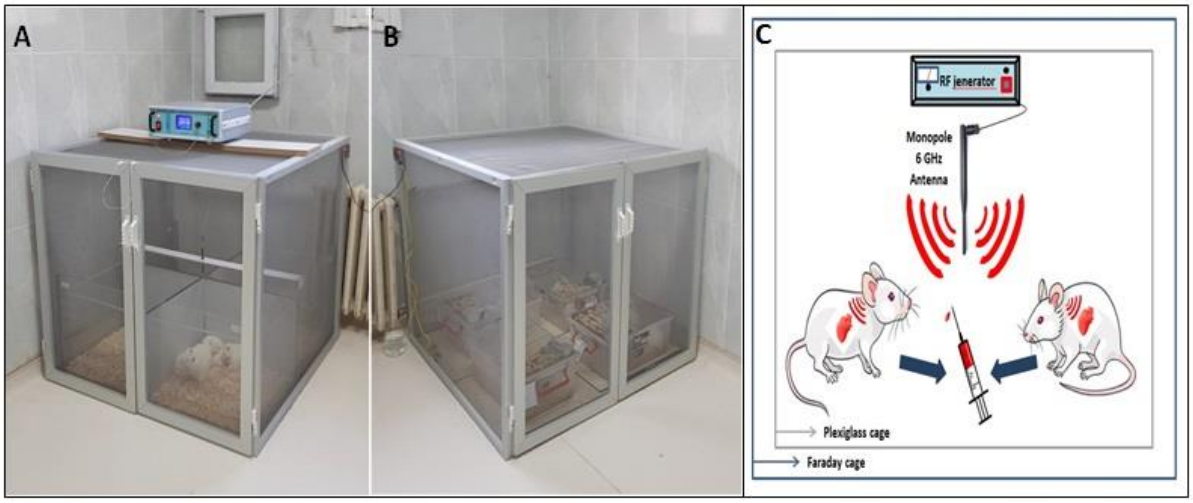


**Figure 2:** Experimental setup and RF generator with 6 GHz RF-EMF applied. A) RF-EMF exposure group, B) Control group, C) Simulation of the top view of the application cage.

After RF-EMF administration, xylazine (mg/kg) and ketamine (mg/kg) injections were administered intraperitoneally to all experimental animals. Blood samples (about 2ml) were taken from rats under anesthesia by cardiac puncture method and transferred to tubes containing EDTA. Then, the EDTA-containing blood tubes were immediately centrifuged in a centrifuge device (Brand; Nuve, Model; NF 1200, Turkey) at 3000 RPM for 30 minutes and their plasma was separated. Plasma samples were transferred to numbered Eppendorf tubes and they were freezed at -200C for 1 hour. Then measurements were taken by ELISA method.

### 2.5. ELISA Appointment Method and Evaluation

The mean plasma levels of pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokines were evaluated by spectrophotometric analysis using the ELISA method. In the study, 96 test subjects performed rat-specific ELISA (TNF-α: SEA133Ra, IFN-γ: SEA049Ra, IL-4: SEA077Ra,

IL-10: SEA056Ra), (Wuhan USCN Business Co., Ltd. | USCN life science KIT INC., China) kit was used. In our study, the Sandwich ELISA method working procedure (Wuhan USCN Business Co., co. USCN life science KIT INC., China) was used to evaluate the results.

## 3. Statistical Analysis

Numerical measurements were summarized as mean and standard deviation (median and minimum-maximum where necessary). Independent Samples t-test was used to compare two dependent numerical measurements that show normal distribution while Mann-Whitney U test was used to compare two dependent numerical measurements that do not show normal distribution. IBM SPSS Statistics Version 20.0 package program was used for statistical analysis of the data (SPSS reference: IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.). The statistical significance level was taken as 0.05 in all tests.

## 4. Results

In this study, cytokine levels in the blood plasma of rats exposed to 6 GHz (0.083 W/kg SAR) RF-EMF were examined in two groups: Control and RFR group. In plasma, pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokine levels were measured as pg/mL (Figure 3). 6 GHz RF-EMF exposure caused significant effects on both pro-inflammatory and anti-inflammatory cytokine levels.

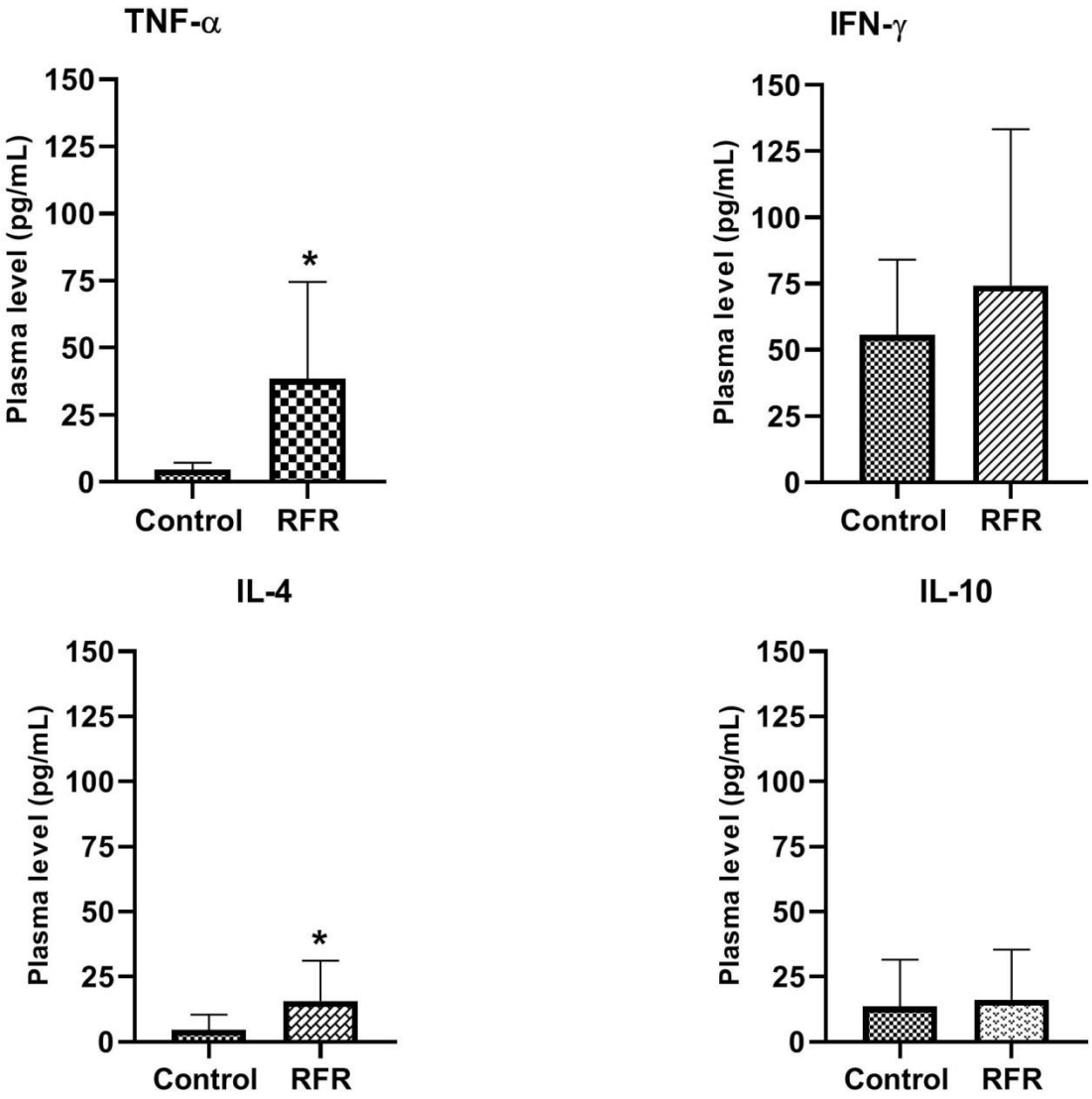


**Figure 3:** Changes of plasma levels of pro-inflammatory (TNF-α, IFN-γ) and anti-inflammatory (IL-4, IL-10) cytokines (n=10). Data are shown as mean ± standard deviation. *, statistically significant ($p < 0.05$).

When the cytokine findings related to the ELISA method were examined; a statistically significant increase was determined in the mean plasma TNF-α level when the Control group (4.7 ± 2.5 pg/mL) was compared with the RFR group (38.5 ± 36.0 pg/mL) ($p=0.008$). The mean plasma IFN-γ level was determined to be not statistically significant difference in the Control group (55.7 ± 28.5 pg/mL) compared to the RFR group (74.4 ± 59.0 pg/mL) ($p>0.05$). On the other hand, a statistically significant increase was determined in the mean plasma IL-4 level when the Control group (4.7 ± 5.8 pg/mL) was compared with the RFR group (15.6 ± 15.5 pg/mL) ($p=0.03$). Similarly, plasma IL-10 level was found to be 13.6 ± 17.9 pg/mL in the Control group and 16.2 ± 19.3 pg/mL in the RFR group. When the mean plasma IL-10 concentrations were compared between the two groups, no

statistically significant difference was detected (p>0.05).

## 5. Discussion

Even though the diversity, intensity and geographical distribution of the use of both cell phone and man-made RF-EMF sources are increasing worldwide, there are some limitations in studies by using these devices in human and experimental animal models. The reason for this situation, various factors such as the inability to create exactly the same environment with the exposure to RF-EMF emitting sources in people's normal lives, significant differences in the sizes of human and experimental animals, and differences in posture have been suggested [19,20]. In our study, when the effect of 6 GHz RF-EMF application on pro-inflammatory cytokine levels was examined in rats, it was observed that TNF-α levels and IFN-γ levels in the RFR group increased compared to the control group. When TNF-α levels were compared between the two groups, the differences were found to be statistically significant (p<0.05). However, when IFN-γ levels were compared between the two groups, no statistically significant difference was found (p>0.05).

In the literature, exposure operations to RF-EMFs below the frequency of 6 GHz have mostly been performed. In this direction, the responses of RF-EMF exposure at different frequency values (900-1800-2450 MHz) on pro-inflammatory cytokines were investigated. Rats were exposed to microwave (MW) radiation at frequencies of (n=24) 900 MHz (0,59 mW/kg SAR), 1800 MHz (0,58 mW/kg SAR) ve 2450 MHz (0,66 mW/ kg SAR) for 2 hours/day and 5 days/week for 60 days. In rats exposed to MW in hippocampal supernatants, it has been reported that there is a significant increase in TNF-α and IFN-γ levels in the 2450 MHz group compared to their values in the 900 and 1800 MHz groups [21]. In another study, the combined effect of 2.45 GHz (0.4060 W/kg SAR) RF-EMF with fine and coarse black carbon (BC) particles was investigated. It was analyzed whether the RAW 264.7 cell line caused cell damage and inflammatory response in macrophages. It has been reported that it causes an increase in TNF-α through the combined effect of EMF and BC at the 24th hour of the study and accelerates apoptosis by activating it [22]. In another study, 1966.1 MHz (2 ve 0.36 W/kg SAR) RF-EMF exposure was reported to cause a statistically significant increase in circulating TNF-α levels [23].

Similarly, in our study, 6 GHz RF-EMF (0.083 W/kg SAR) was observed to significantly increase the synthesis of pro-inflammatory TNF-α cytokine levels. However, we was observed a non-statistically significant increase in the synthesis of pro-inflammatory IFN-γ cytokine levels. According to our findings, it can be said that the immune system can be affected by the 6 GHz RF-EMF effect and the expression of pro-inflammatory cytokines can be supported. On the other hand, it is present in studies report that RF-EMFs does not have a significant activation in the induction of pro-inflammatory cytokines [15,24]. In this regard, in a study conducted as part of the in-vitro effects of 1950 MHz (1mW/g SAR) RF-EMF on human immune cells, exposure was performed on human blood cells in intermittent mode and for a period of 8 hours. It has been reported that it does not cause positive/negative effects on intracellular TNF-α and IFN-γ levels [24]. On the other hand, it has been reported that TNF-α and IFN-γ levels decreased significantly compared to the control group under 2450 MHz (0,478 W/kg SAR) RF-EMF exposure applied using a mouse model [15].

In our study, it is suggested that the reason for the increases in TNF-α and IFN-γ levels may be due to the dependence on the frequency and exposure time of the applied RF-EMF. On the other hand, it suggests that the increase in TNF-α and IFN-γ levels may not be directly and solely due to the effect of RF-EMF, but that emotional/oxidative stress caused by these sources may also contribute. Because, these results we obtained have been shown to be supported by the report that the immune system affects oxidative stress markers [25].

Because there are various environmental factors (EMF, noise, light/dark, heat, etc.) in the RF-EMF interaction, it can be effective in the development of stress-related behaviors in subjects. In this direction, it has been reported in a study focusing on the potential harms caused by RF-EMFs from mobile phones that they can lead to a large number of health problems [26]. From these results, the immune system modulating effect of the interactions of pro-inflammatory cytokines with RF-EMF is controversial.

On the other hand, in our study, when the effect of 6 GHz RF-EMF (0.083 W/kg SAR) application on anti-inflammatory cytokine levels was examined in rats, it was observed that the mean plasma concentrations of IL-4 and IL-10 in the RFR group increased compared to the control group. When concentrations of IL-4 were compared between the two groups, the differences were found to be statistically significant (p<0.05). However, when concentrations of IL-10 were compared between the two groups, no statistically significant difference was found (p>0.05). Our data show that following 6 GHz RF-EMF exposure, it does cause significant effects on anti-inflammatory responses. There are several studies that support our study [27,28]. In contrast to our study, it has been shown in some studies that RF-EMFs do not cause any changes in IL-4 and IL-10 levels [29,30].

In a study conducted in this direction, it was reported that there was no change in serum IL-4 protein concentrations in a study conducted to determine the effect of 2.14 GHz (0,2 W/kg SAR) RF-EMF on the T cell [31]. In another study, no statistically significant changes in serum IL-4, IL-10 levels and serum TNF-α and IFN-γ levels of rats exposed to RF-EMF were detected between the groups [32]. In order to determine the effects of RF-EMF on T helper cells, the possible effects of 900 MHz (0,13 W/kg SAR) RF-EMF on peripheral blood mononuclear cell (PBMC) cultures at different durations (15, 30, 45, and 60 minutes) and at two different distances (0 to 5 cm) were investigated. It has been observed that IL-10 increased initially in exposed cells, but decreased depending on the duration of exposure and reached values close to the control group. From these results, it was concluded that the effect of RF-EMF on the percentage of IL-10 is temporary [33].

These results indicate that RF-EMFs do not have important roles in the expression of anti-inflammatory cytokines and the regulation of the physiological function of inflammatory reactions. However, the immune system modulating effect of interactions of anti-inflammatory cytokines with RF-EMF is

controversial. Because it has been reported by some researchers that exposure to RF-EMF can cause negative effects on anti-inflammatory cytokine levels [34,35].

In another study conducted in this direction, it was reported that extremely low frequency (50 Hz) EMF had a significant decrease in rats exposed to 1, 100, 500, and 2000 μT different magnetic flux densities IFN-γ and IL-4 serum levels only 100 μT [36]. In another study, the modulatory effects of low-frequency (50 Hz) EMF on the production of anti-inflammatory cytokines were investigated. It has been stated that exposure for 2, 3, and 6 hours results in a time-dependent decrease in the concentration of IL-10 cytokine protein [37].

In studies carried out to date, exact results have not yet been obtained in determining the effects of exposure to RF-EMF sources applied using different procedures and methods on the immune system [20,38]. We think that the reason why there was change in anti-inflammatory cytokine levels in the study may be due to the fact that the applied SAR value (0.083 W/kg) complies with the values given by the ICNIRP (International Commission for Non-ionizing Radiation Protection) [18].

## 6. Conclusion

In studies conducted on the effects of RF-EMF exposure on the immune system, results in the same direction can be obtained, as well as opposite results appear. In our study, although 6 GHz RF-EMF (0.083 W/kg SAR) exposure caused significant changes in TNF-α and IL-4 cytokine levels between groups, it did not cause any effect on the deficiency of IFN-γ and IL-10 cytokine levels. However, the ability of the immune system to perform its functions in a certain order depends on the balanced distribution of pro-inflammatory and anti-inflammatory cytokines within itself. That is, when the expression of pro-inflammatory cytokines increases, the inhibition of anti-inflammatory cytokines should increase accordingly. However, our data show that anti-inflammatory cytokines such as IL-4 and IL-10 do not have a strong interaction with pro-inflammatory cytokines such as TNF-α and IFN-γ. We believe that more advanced experimental and epidemiological studies are needed on the immune system in order to better and fully understand the health and safety effects that RF-EMFs may cause in living beings as a result of increasing exposure intensity.

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