# THE EFFECT OF 900 MHZ RADIOFREQUENCY (RF) RADIATION ON SOME HORMONAL AND BIOCHEMICAL PARAMETERS IN RABBITS.

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## Abstract

In this study, the effect of cell phone frequency 900 MHz radiofrequency (RF) radiation on rabbits' some hormonal and biochemical parameters was investigated. 14 male, adult, New Zealand albino rabbits were separated into two groups as control and experimental (n=7). For the experimental group, rabbits exposed to 900 MHz frequency RF radiation 2 h per day (7 days in a week) for 90 days. Control group rabbits' procedure was the same as experimental group except the exposure of RF. Before the exposure, at first and third month of exposure; Glucose, Creatinin, Na, K, Cl, Calcium, Phosphorus, Total protein, Albumin, Globulin and Magnesium levels and T3, T4, TSH, LH, FSH, Testesteron, Estradiol, Prolactin, Cortisol, ACTH and Growth Hormon levels were determined from rabbits' blood which was taken from V.Marginalis in their right ear.

There was not any significant difference in neither hormonal nor biochemical parameters between experimental and control groups before exposure ($p>0.05$). At first month of RF exposure; there was not any significant difference in biochemical parameters ($p>0.05$). However, T4, Testesterone, Estradiol, Cortisol and ACTH levels increased but this increasing was not stastistically significant ($p>0.05$). After third month exposure, it was found that there was not any significant difference in biochemical parameters ($p>0.05$), but there was an increase at T4, Cortisol and ACTH levels and only increasing in ACTH levels was stastistically significant ($p<0.05$).

It was concluded that 900 MHz RF exposure did not change some important biochemical and hormonal parameters except ACTH levels after 90 days exposure.

(*Journal of International Dental and Medical Research 2009; 2: (3), pp. 110-115)*

**Key words:** Mobile phone, Radiofrequency Radiation. ACTH, FSH,LH, Glucose, Albumin

**Received date: 23 June 2009** **Accept date: 18 October 2009**

## Introduction

The exponential increase in the use of mobile phones is raising major concerns on possible consequences of the interaction between radiofrequency (RF) and biological systems (1). Also some of the international institutes and groups have tried to determine the possible risks by establishing the limited estimations of this radiation (2,3). They have been using calculated or observed thermal effects to determine these limitations, but do not consider possible effects of chronic or repeated nonthermal exposures (4). However, nonspecific neurovegetative syndromes such as physical and neural asthenia, sleep disorders, humoral changes, headache and myalgia have been observed in males exposed to chronic low intensity RF radiation (5).

It was shown with animal studies that exposure to RF radiation had effects on the endocrine or the nervous systems and especially the thyrotropin (TSH) secretion (5). In addition, Navakatikian and Tomashevyskaya have determined inhibition in testesteron and insuline secretion in rats which are exposed to low intensity microwaves (6). Seze et al. reported that one month of intermittent exposures to RF radiation from a cellular telephone does not induce a long-lasting or cumulative effect on the hormone secretion rate of the anterior pituitary gland in humans (5).

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Braune et al. found that RF radiation emitted from mobile phone did not affect to the serum norepinefrin, epinefrin, cortisol and endotelin amounts of healthy people (7). In their study Rajkovic et al. have exposed to low-frequency electromagnetic fields to male rats during three months and afterwards they investigated the hormonal and functional structures of thyroid tissues (8). The results obtained from this study showed that exposure of low-frequency electromagnetic fields to male rats for 3 months have caused morphofunctional alteration on thyroid tissues.

Mann et al. have investigated the effects of pulsed high-frequency electromagnetic fields that emitted from circular polarized antenna, on neuroendocrine system of healthy peoples (9). The hormones investigated in this study are Growth Hormone (GH), Cortisol, Luteinizing Hormone (LH) and melatonin hormone (9). The results obtained in this study determine that low intensity high-frequency electromagnetic fields emitted from mobile phone causes no effects on hormone secretion other than only a slight increase in cortisol formation (9). It has been reported that radio frequency radiation at mobile phone frequency exposed to rodents and human has changed many parameters related to stress. Stagg et al. who investigated this potential relationship, studied the effect of 1.6 GHz puls-modulated RF radiation on Fischer 344 rats' Adrenocorticotropin (ACTH), corticosterone and brain ornithine decarboxylase (ODC) levels (10). At the end of the study, these investigators had reported that puls-modulated RF fields (up to 5 W/kg SAR level) did not indicate any stress response related to ACTH, corticosteroid and brain ODC levels. Radon et al. reported that the salivary concentrations of melatonin and cortisol did not change after 900 MHz RF exposure in healthy male students (11).

The contradictions of the studies done on both human and experimental animals relating to the effect of RF radiation emitted from mobile phones on endocrine system, directed us to make such a study. In the present study, the results obtained from the long term chronic exposures will enlighten the contradictions about the potential effects of low intensity RF radiation. The main aim of our study is to investigate whether the RF radiation emitted by mobile phones, which their usage rapidly increase both in our country and in the world, has any effects on endocrine system and some biochemical parameters, and to help to enlighten the contradictions about the subject.

## Materials and Methods

### Subjects and Animal Care

The experiments were performed on 14 adult male New Zealand rabbits obtained from Medical Science Application and Research Center of Dicle University, aged 3- 4 months, weighing 2500- 3500g, and fed with standard pelleted food (TAVAS Inc. Adana, Turkey) and tap water. The animals were kept in 14/10h light/dark environment at constant temperature of 22 ± 3°C, 45 ± 10% humidity and one rabbit in one cage. 14 rabbits were separated into two groups such as control (n=7) and experimental (n=7). All animal procedures were in agreement with the principles of laboratory animal care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center (Protocol Number; 2005/29).

### 900 MHz RF Exposure and Experimental Method

Rabbits are placed in the radial mode, in cages (31×17.5×17) cm in size with opening and closing wood in the top, the front part bended and has 7 cm diameter circular opening that rabbits neck portion can pass through (Figure 1).

**Figure 1.** Experimental setup.

RF radiation obtained from 900 MHz frequency RF generator (GSM Simulator 900PM10 type, Everest Comp., Adapazarı, Turkey) exposed to 7 rabbits in experimental group for 90 days two hours a day, 7 days a week. The antenna of RF generator is placed in a plastic cylindrical line so that all rabbits can expose to RF equally (Figure 1). The properties of RF signal obtained from the RF generator are: Frequency: 900 MHz, Output Power: 2 W, modulation frequency 217 Hz, pulse width: 577 μs. The power density of RF radiation obtained from the antenna were detected with an electromagnetic field meter (EMR-300, Narda, Pfullingen, Germany) on different regions of rabbit (Figure 1). For control group, the rabbits were placed into the wooden

cages (2 h/day/7day in a week for 90 days), same procedure were applied to the rabbits, except that RF generator was turned off.

To determine the effects of RF radiation on some biochemical and hormonal parameters of rabbits, the blood samples of experimental and control groups collected three times from marginal ear vein with intravenous canulla depended of rabit's size. The blood samples were taken before, immediately after first and third months of RF radiation exposure. Serum was removed from blood via centrifugation for 5 minutes at 6500 rpm.

**Determination of Biochemical Parameters**

For the biochemical parameters used in the study; Glucose, Creatinine, Na, K, Cl, Calcium, Phosphorus, Total Protein, Albumin, Globulin and magnesium values were determined by using the enzymatic method by Abbot Aeroset Autoanalyzer (USA) which is provided from Central Laboratory of Faculty of Medicine in Dicle University. The original kit of Abbot companies was used in the study.

**Determination of Hormonal Parameters**

For the hormonal parameters used in the study; T3, T4, TSH, LH, FSH, Testesterone, Estradiol, Prolactin, Cortisol, ACTH and growth hormone values were determined by electrochemiluminescence method using Roche Diagnostic E-170 (Germany) which is provided from Central Laboratory of Medicine Faculty of Dicle University. The original Roche kit was used in study.

**Statistical Analysis**

Mean values and standard deviations were calculated, and statistical significance of the differences between exposure and control groups was evaluated. A computer program (SPSS 10.0, SPSS Inc., Chicago, IL, USA) were used for statistical analysis. Data were analyzed by Mann-Whitney U test which is a nonparametric test. All hypothesis tests used a criterion level of p = 0.05.

**Results**

As a result of experimental study done; before the exposure there was not any statistically significant differences found between the experimental and control group rabbits' biochemical parameters such as Glucose, Creatinine, Na, K, Cl, Calcium, Phosphorus, Total Protein, Albumin, Globulin and Magnesium (Table 1). Also in analysis of biochemical parameters done after first month of RF exposure; there was not any statistically significant difference between the experimental and control groups (Table 2).

| Parameters | Control Groups Mean± S.D | Exp. Groups Mean± S.D | U | P |
|---|---|---|---|---|
| Glucose (mg/dL) | 130.428±8.100 | 129.0±19.328 | 20.0 | p>0.05 |
| Creatine (mg/dL) | 1.70±0.251 | 1.571±0.149 | 18.0 | p>0.05 |
| Na (mmol/L) | 136.142±15.973 | 136.571±15.757 | 22.5 | p>0.05 |
| K (mmol/L) | 4.157±0.756 | 4.414±0,715 | 20.0 | p>0.05 |
| Cl (mmol/L) | 104.428±1,618 | 104.285±2.430 | 23.0 | p>0.05 |
| Ca (mg/dL) | 14.428±0.807 | 14.728±0.786 | 21.0 | p>0.05 |
| P (mg/dL) | 3.471±0.969 | 3.957±0.531 | 15.5 | p>0.05 |
| T.Protein (g/dL) | 5.100±0.369 | 5.100±0.216 | 17.5 | p>0.05 |
| Albumin (g/dL) | 1.742±0.139 | 1.785±0.146 | 19.5 | p>0.05 |
| Globulin (g/dL) | 3.342±0.350 | 3.328±0.197 | 19.5 | p>0.05 |
| Mg (mg/dL) | 2.714±0.186 | 2.757±0.276 | 22.5 | p>0.05 |

**Table 1.** The statistical analyses of some biochemical parameters in rabbits before 900 MHz RF exposure. The values represent the mean ±S.D (Standard Deviation), Exp: Experimental.

| Parameters | Control Groups Mean± S.D | Exp. Groups Mean± S.D | U | P |
|---|---|---|---|---|
| Glucose (mg/dL) | 124.571±14.942 | 141.857±16.385 | 9.5 | p>0.05 |
| Creatine (mg/dL) | 1.528±0.095 | 1.500±0.129 | 21.5 | p>0.05 |
| Na (mmol/L) | 141.0±1.291 | 141.571±1.988 | 21.0 | p>0.05 |
| K (mmol/L) | 3,751±0.190 | 3.885±0.429 | 20.0 | p>0.05 |
| Cl (mmol/L) | 110.0±0.816 | 110.285±0.755 | 19.5 | p>0.05 |
| Ca (mg/dL) | 14.257±0.694 | 14.385±0.691 | 22.0 | p>0.05 |
| P (mg/dL) | 4.114±0.491 | 4.0±0.503 | 22.5 | p>0.05 |
| Total Protein (g/dL) | 5.742±0.482 | 5.771±0.303 | 23.5 | p>0.05 |
| Albumin (g/dL) | 1.814±0.146 | 1.828±0.197 | 21.5 | p>0.05 |
| Globulin (g/dL) | 3.914±0.371 | 3.942±0.276 | 24.0 | p>0.05 |
| Mg (mg/dL) | 1.914±0.177 | 1.885±0.211 | 22.5 | p>0.05 |

**Table 2.** The statistical analyses of some biochemical parameters in rabbits after first month of 900 MHz RF exposure. The values represent the mean ±S.D (Standard Deviation), Exp: Experimental.

When evaluating the findings after 3 months RF exposure; again it was determined that there was not any significant difference regarding biochemical parameters (Table 3).

In the present study it was attempted to be determine the changes on hormonal parameters

| Parameters | Control Groups Mean± S.D | Exp. Groups Mean± S.D | U | P |
|---|---|---|---|---|
| Glucose (mg/dL) | 125.285±11.785 | 121.571±7.807 | 21.50 | p>0.05 |
| Creatine (mg/dL) | 1.485±0.267 | 1.471±0.160 | 23.0 | p>0.05 |
| Na (mmol/L) | 141.285±2.984 | 143.142±1.676 | 15.0 | p>0.05 |
| K (mmol/L) | 4.442±0.304 | 4.5±0.365 | 24.0 | p>0.05 |
| Cl (mmol/L) | 106.428±1.988 | 106.571±3.359 | 20.5 | p>0.05 |
| Ca (mg/dL) | 14.228±0.354 | 14.200±0.365 | 23.5 | p>0.05 |
| P (mg/dL) | 3.428±0.471 | 3.5±0.424 | 22.0 | p>0.05 |
| T.Protein (g/dL) | 5.571±0.298 | 5.628±0.213 | 22.5 | p>0.05 |
| Albumin (g/dL) | 1.785±0.106 | 1.828±0.075 | 19.0 | p>0.05 |
| Globulin (g/dL) | 3.785±0.254 | 3.828±0.197 | 21.5 | p>0.05 |
| Mg (mg/dL) | 1.642±0.139 | 1.700±0.163 | 22.0 | p>0.05 |

**Table 3**. The statistical analyses of some biochemical parameters in rabbits after third month of 900 MHz RF exposure. The values represent the mean ±S.D (Standard Deviation), Exp: Experimental.

such as T3, T4, TSH, LH, FSH, Testosterone, Estradiol, Prolactin, Cortisol, ACTH and growth hormone, after the RF exposure. In evaluation done before the exposure, it was found that there was not any significant change in hormonal parameters of experimental and control group of rabbits (Table 4).

| Parameters | Control Groups Mean± S.D | Exp. Groups Mean± S.D | U | P |
|---|---|---|---|---|
| T-3 (ng/mL) | 1.044±0.191 | 0.939±0.172 | 18.0 | p>0.05 |
| T-4 (ug/mL) | 3.731±0.395 | 3.597±0.544 | 21.5 | p>0.05 |
| TSH (ulU//mL) | 0.0073±0.001 | 0.007±0.001 | 21.5 | p>0.05 |
| LH (ulU//mL) | 0.100±0.00 | 0.100±0.00 | 24.5 | p>0.05 |
| FSH (ulU//mL) | 0.102±0.005 | 0.100±0.00 | 21.0 | p>0.05 |
| Testesterone (ng/mL) | 0.506±0.283 | 0.521±0.226 | 23.0 | p>0.05 |
| Estradiol (pg/mL) | 17.342±1.237 | 16.472±1.204 | 14.0 | p>0.05 |
| Prolactine (ng/mL) | 0.470±0.00 | 0.470±0.00 | 24.5 | p>0.05 |
| Cortizol (ug/dL) | 1.258±0.548 | 1.279±0.550 | 24.0 | p>0.05 |
| ACTH (pg/mL) | 15.208±5.796 | 16.228±6.658 | 24.0 | p>0.05 |
| Growth Hormone (ng/mL) | 0.054±0.005 | 0.053±0.004 | 21.0 | p>0.05 |

**Tablo 4.** The statistical analyses of some hormonal parameters in rabbits before 900 MHz RF exposure. The values represent the mean ±S.D (Standard Deviation), Exp: Experimental.

| Parameters | Control Groups Mean± S.D | Exp. Groups Mean± S.D | U | P |
|---|---|---|---|---|
| T-3 (ng/mL) | 1.008±0.116 | 0.981±0.223 | 23.0 | p>0.05 |
| T-4 (ug/mL) | 2.810±0.394 | 3.284±0.814 | 16.0 | p>0.05 |
| TSH (ulU//mL) | 0.0076±0.001 | 0.0067±0.001 | 17.0 | p>0.05 |
| LH (ulU//mL) | 0.100±0.00 | 0.100±0.00 | 24.5 | p>0.05 |
| FSH (ulU//mL) | 0.100±0.00 | 0.100±0.00 | 24.5 | p>0.05 |
| Testesterone (ng/mL) | 0.942±0.393 | 1.808±1.07 | 13.0 | p>0.05 |
| Estradiol (pg/mL) | 18.804±2.234 | 22.425±7.778 | 17.0 | p>0.05 |
| Prolactine (ng/mL) | 0.470±0.00 | 0.470±0.00 | 24.5 | p>0.05 |
| Cortizol (ug/dL) | 0.239±0.195 | 0.253±0.119 | 22.0 | p>0.05 |
| ACTH (pg/mL) | 9.905±3.786 | 10.682±6.11 | 24.5 | p>0.05 |
| Growth Hormone (ng/mL) | 0.0510±0.003 | 0.05±0.00 | 21.0 | p>0.05 |

**Table 5.** The statistical analyses of some hormonal parameters in rabbits after first month of 900 MHz RF exposure. The values represent the mean ±S.D (Standard Deviation), Exp: Experimental.

At the end of first month of exposure, it was found that there was an increase in T4, Testosterone, Estradiol, Cortisol and ACTH levels that is not statistically significant (Table 5). As for analysis done on bloods taken from rabbits after 3 months exposure, there was an increase at T4, Cortisol and ACTH levels and only increasing in ACTH levels was stastistically significant ($p<0.05$)(Table 6).

Values of average power density of RF radiation obtained from RF generators measured at different levels of rabbits' bodies and they were as follows; at the level of rabbits' nose 0.215 mW/cm$^2$, at the level between two eyes 0.118 mW/cm$^2$, at the level between two ears 0.114 mW/cm$^2$, at the level of back 0.026 mW/cm$^2$, at the level of rear leg 0.013 mW/cm$^2$. The measured background RF level was equal to 0.0001 mW/cm$^2$ when the RF generator is turned off.

## Discussion

In the present study, biochemical and hormonal effects of 900 MHz frequency RF radiation emitted from mobile phones were evaluated by determining the levels of some hormone and biochemical parameters. Also in this present study; to determine the cumulative effect of RF radiation depending on the time, changes at parameters studied were determined before

| Parameters | Control Groups Mean± S.D | Exp. Groups Mean± S.D | U | P |
|---|---|---|---|---|
| T-3 (ng/mL) | 1.155±0.055 | 1.143±0.129 | 24.5 | p>0.05 |
| T-4 (ug/mL) | 3.874±0.546 | 4.091±0.673 | 22.0 | p>0.05 |
| TSH (ulU//mL) | 0.0087±0.001 | 0.0083±0.001 | 19.5 | p>0.05 |
| LH (ulU//mL) | 0.100±0.00 | 0.100±0.00 | 24.5 | p>0.05 |
| FSH (ulU//mL) | 0.103±0.007 | 0.100±0.00 | 17.5 | p>0.05 |
| Testesterone (ng/mL) | 1.307±0.876 | 0.851±0.552 | 19.0 | p>0.05 |
| Estradiol (pg/mL) | 32.900±5.954 | 32.548±8.764 | 18.0 | p>0.05 |
| Prolactine (ng/mL) | 0.470±0.00 | 0.470±0.00 | 24.5 | p>0.05 |
| Cortizol (ug/dL) | 0.758±0.280 | 1.090±0.374 | 12.0 | p>0.05 |
| ACTH (pg/mL) | 13.467±4.245 | 22.930±12.323 | 8.0 | p<0.05 |
| Growth Hormone (ng/mL) | 0.069±0.003 | 0.069±0.015 | 20.0 | p>0.05 |

**Table 6.** The statistical analyses of some hormonal parameters in rabbits after third month of 900 MHz RF exposure. The values represent the mean ±S.D (Standard Deviation), Exp: Experimental.

exposure to RF, at first month of exposure and after third month of exposure. When biochemical data obtained was evaluated; although, there was increase and decrease in some variables after first and third month of exposure, it was determined that these changes were not significant. The hormones secreting from endocrine system are the important variables that regulate the metabolic activity. Therefore, it was investigated whether chronic exposure of 900 MHz RF radiation can affect to the important hormonal parameters such as T 3, T 4, TSH, LH, FSH, testosterone, estradiol, prolactin, cortisol, ACTH and growth hormone. In the present study, it was determined that; in first month of exposure; T 4, testosterone, estradiol, cortisol and ACTH levels were increased, but this increasing was not significant, however; after 3 months exposure, T 4, cortisol and ACTH levels were also increased, but from these changes, only an increasing in ACTH levels were significant.

Endocrine system of mammals regulate the body functions to protect the homeostasis in cases of basal and stress situations (12). As RF radiation can be taken into account as a type of environmental stress, it is important to determine the effect of RF radiation emitted from mobile phones which are intensively used nowadays, on endocrine system and on the biochemical parameters, which are important for the functioning of biological systems. Different results are encountered in studies done regarding the effects of microwave and RF radiation, at different frequencies and different power densities, on hormones secreted by the endocrine system. Michaelson et al. found that GH level was decreased in rats which exposed to 2450 MHz frequency, 36 mW/cm$^2$ power density microwave radiation for 60 minutes (13). These researchers observed that the decreasing in GH levels is associated with mean body temperature increasing up to 40.9 °C and making a peak. Lu et al. concluded that corticosterone and prolactin concentrations increased after the exposure of 2.45 GHz frequency amplitude modulated microwave radiation (14). Lotz and Podgorski measured the plasma cortisol, GH and T 4 levels in Rhesus monkey which were exposed to 1.29 GHz microwave radiation (15). These authors reported that GH and T 4 concentrations did not change but cortisol levels were increased during the exposure of 38 mW/cm$^2$ power density RF radiation. Lu et al. concluded that the change in corticosterone, thyrotropin and GH levels can be correlated with power density or colon temperature of rats exposed to microwave for 1 hour (16). Some researchers observed an increase in plasma corticosterone levels in animals which were exposed to Radiofrequency Radiation (RFR) at the level of SAR (Specific Absorbtion Rate) that creates thermal stress (13,17,18-20). Liburdy suggested that thermal stress which formed as a result of whole body RFR exposure stimulated the release of adrenal steroids in blood (20.21). However, it has been reported that, adrenocortical inhibition occurs at SAR levels lower than 4 W/kg without a significant increase in colon temperature (19). Akdag et al. determined that 9450 MHz RF exposure can cause to significant decrease in GH and FSH levels and increase in estradiol levels of female rats (22).

In the present study, at the first month of RF exposure, non significant increases in T4, testosterone, estradiol, cortisol and ACTH levels were determined but after third months of RF exposure T 4, cortisol and ACTH levels increased and only the increase in ACTH levels were significant. It has been proposed that low intensity microwave radiation can cause stress (4). Hormones like ACTH and cortisol are such hormones that generally associated with stress but GH and prolactin are hormones which is less associated with stress (4). In our study, the increasing occurred in cortisol and ACTH levels at the first and third months of the exposure can be due to stress induced by low-intensity RF radiation which is a stress factor.

The results obtained in our study regarding the hormonal parameters are consistent with the data of the studies that investigate the effects of RF and microwave radiation on the neuroendocrine system (5,7,9,10). In the present study, there was not any changes in biochemical parameters which have important functions on the biological system, after RF exposure at both 1 month and 3 months. Therefore, it can be suggested that RF radiation obtained in our current experimental conditions are not enough to affect the biochemical parameters in rabbits. However, much longer-term researches that will be done on both experimental animals and human will help to answer the questions existing on this issue.

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