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Effects of Radiofrequency Radiation by 900 MHz Mobile Phone on Periodontal Tissues and Teeth in Rats

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Abstract: The aim of this study, is to research an answer to the question of have mobile phones effects on periodontal tissues and teeth. Twenty eight Wistar Albino adult male rats (4 months) with initial average weight of 267±15 g were obtained from the Medical Science Application and Research Center, caged individually and fed with standard pelletted food (TAVAS Inc. Adana, TR). They were separated into three groups such as cage control (n = 7), sham exposed (n = 7) and experimental (n = 14) and kept on a 14/10 h light/dark schedule. The rats were confined in a Plexiglas carousel and rat heads in the carousel exposed to 900 MHz microwave exposure emitted from generator. For the study group, rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. Although, there was no statistical difference determined among the groups of experimental and sham groups ($p>0.05$), there were numerically more individuals effected of RF radiation in the experimental group. In our study is the statistically significant difference among the groups of experimental, sham and control groups in the case of vasodilatation in periodontal ligament and alveolar bone ($p<0.05$). By the histopathologic evaluation abnormal changes like vasodilatation and focal bleeding areas were determined in periodontal ligament, alveolar bone, gingiva and pulpa among some individuals. These degenerations were in different levels. These findings of our study indicates that there is need to more animal, human being and epidemiologic studies including much more individuals.

Key words: GSM mobile phone, radiofrequency radiation, rats, periodontal tissues, teeth

INTRODUCTION

Radiofrequency (RF) fields are part of the electromagnetic spectrum. For the purpose of international electromagnetic fields (EMF) project, such fields are defined as those within the frequency range 10 MHz and 300 GHz. Natural and human-made sources generate RF fields of different frequency. Common sources of RF fields include: FM radio mobile telephones, television broadcast, microwave ovens, medical diathermy, radar, satellite links, microwave communications and sun (Fact Sheet, 1998).

Mobile telephones, some times called cellular phones (GSM, Global System for Mobile Communication) or handies, are now an integral part of modern telecommunications. In some parts of the world, they are the most reliable or only phones avaliable. In others, mobile phones are very popular because they allow people to maintain continuous comminication without hampering freedom of movement (Fact Sheet, 2000).

The widespread use of mobile phones in recent years has raised many questions about whether their use is safe, because the operator is exposed to Electromagnetic Waves (EMW) in Ultra High-Frequency (UHF) range

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(i.e., 300-3000 MHz). The exposure to high-intensity EMW in the UHF range produces heat and can cause thermal damage to the brain. However, the effects of exposure to EMW of sufficiently low-intensity not to cause elevation of brain temperature are yet to be clarified (Tsurita *et al*., 2000). The use of mobile phones operating in the 900 MHz frequency band is very widespread and ever increasing (Maes, 2001). Conflicting effects of EMF have been reported in Electroencephalogram (EEG). While, some studies have reported that exposure to EMF around 900 MHz have had effects on the EEG (Mann and Roschke,1996; Eulitz *et al*., 1998; Freude *et al*., 1998; Krause *et al*., 2000) not all studies have been able to demonstrate such effects (Röschke and Mann, 1997; Wagner *et al*., 1998).

Concern has been expressed for number of years that exposure to RF fields emanating from mobile phones and radar and television transmitters may increase the incidence of cancer in humans. Epidemiological studies have not indicated an increased cancer risk, but the methodology and exposure assessments are generally considered to have been subpotimal (NRPB, 1992; UNEP/IRPA/WHO, 1993; Repacholi, 1996).

Experiments rewieved for the World Health Organization (UNEP/IRPA/WHO, 1993) and for the National Radiological Protection Board of the UK (NRPB, 1992) did not demonstrate convincingly any direct damage to DNA after acute or chronic exposure of biological sytems to RF fields. In particular, when temperatures were maintained within normal physiological limits, no evidence for induction of DNA breaks or chromosome aberrations was found. On the other hands, two recent studies have suggested that RF fields can affect DNA (Sarkar *et al*., 1994; Lai and Singh,1982).

The periodontium consists of the investing and supporting tissues of the tooth (gingiva, Periodontal Ligament (PL), cementum, Alveolar Bone (AB). The gingiva is the part of the oral mucosa that covers the alveolar processes of jaws and surrounds the necks of the teeth. The periodontal ligament is the connective tissue that surrouns the root and connects it with the bone. It is continuous with the connective tissue of the gingiva and communicates with the marrow spaces through vascular channels in the bone. Cementum is the calcified mesenchymal tissue that forms the outer covering of the anatomic root. There are two mian types of root cementum: acellular (primary) and cellular (secondary). The alveolar process is the portion of the maxilla and mandible that forms and supports the tooth sockets (alveoli). It forms when the tooth erupts to provide the osseous attachment to the forming periodontal ligament; it disappears gradually after the tooth is lost (Carranza and Newman, 1996).

We have examined all the studies in which the mobile phones effects on health were invesigated. However, there were a lot of studies in that the effects of mobile phones on head were investigated, we didn't encounter any histopathologic study focused on the effects on periodontal tissues or teeth. Because of this we planned this study to investigate the effects of mobile phones on periodontal tissues and teeth histopathologically. The aim of this study, is to research an answer to the question of have mobile phones effects on periodontal tissues and teeth.

MATERIALS AND METHODS

Subjects and animal care: Twenty eight Wistar Albino adult male rats (4 months) with initial average weight of 267±15 g were obtained from the Medical Science Application and Research Center of Dicle University (Diyarbakir, TR), caged individually and fed with standard pelletted food (TAVAS Inc. Adana, TR). Final average weight of the animals was 363±18 g. They were separated into three groups such as experimental (n = 14), sham exposed (n = 7) and cage control (n = 7) and kept on a 14/10 h light/dark schedule. During the study, the ambient temperature (22°C) and relative humidity (45%) were maintained in the normal range for these animals. 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.

Exposure and measurement of radiation: A generator (GSM Simulator 900 PM10 type Everest Comp., Adapazari, Turkey), which is produce 900 MHz radiofrequency radiation used in this study to represent exposure of global systems for mobile communication (GSM) cellular. Emitted power (circular space distribution) of the generator was fixed at 2 W during exposure. Antenna of the generator was equivalent to anterna of mobile phones.

Fig. 1: Exposure system

The rats were confined in a Plexiglas carousel and rat heads in the carousel exposed to 900 MHz microwave exposure emitted from generator (Fig. 1). For the study group, rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. For the sham group, rats were placed into the carousel and same procedure were applied to the rats (2 h/day, 7 days/week, 10 months), except that the generator was turned off. Antenna of the generator was placed at the center of Plexiglas carousel to provide ideal exposure (Fig. 1). Distance of antenna to head of the rats was 1.5 cm. Power Density inside Plexiglas carousel cages was measured by EMR 300 (NARDA, Pfullingen, Germany). Average power density at the center of the carousel cages was measured as 0.0252 mW cm^{-2}. However, average power density at the area of rat tails was as measured 0.0045 mW cm^{-2}.

For the cage control, nothing applied to rats in this group and they completed their life cycle in the cage during the study period. On the last day of the study, immediately after the last exposure, the rats were euthanized with a lethal dose of intraperitoneal pentobarbital and maxillary posterior teeth with the surrounding soft and hard tissues were extripated completely. Tissues was fixed in 10% buffered formalin.

Histo-pathological procedure: Histopathologic evaluation was performed in Dicle University Pathology Department. Tissues was fixed in 10% buffered formalin and fixed in Bouin's fixative. All tissues were processed in paraffin. Consecutive 4 mm thick sections were stained with haematoxylin and eosin and examined microscopically. Histological and pathological assessments were done with a light microscope.

Statiscal methods: Exact test, Pearson Chi-Square (λ^2) Test and Comporision of Proportion Tests were used in analyses. SPSS-15.0 for Windows ve med calc version 9.4.2.0 stathistic software programs were used to make calculations and evaluations.

RESULTS

By the histopathologic evaluation abnormal changes like vasodilatation and focal bleeding areas were determined in periodontal ligament, alveolar bone, gingiva and pulpa among some individuals. These degenerations were in different levels. Because of this, the vasodilatation scores were recorded as no, mild and severe. The abnormal change rates were shown as percentages in tables and the differences among the groups were evaluated statistically.

The vasodilatation rates and comparisions were shown in Table 1, focal bleeding areas comparision in Table 2. There were no changes in cement, dentin and enamel tissues among all the groups, because of this these tissues are not shown in tables.

In Table 1 the tissues vasodilatation percentages were shown. Although, there was no statistical difference between the control and sham groups among all tissues

Table 1: The comparision of the groups in the aspect of vasodilatation among the evaluated tissues

	Periodontal ligament			Alveaolar bone			Gingiva			Pulp		
Groups	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe
Group n = 14	35.7% (n = 5)	57.1% (n = 8)	7.1% (n = 1)	64.3% (n =9)	28.6% (n = 4)	7.1% (n = 1)	85.7% (n = 12)	14.3% (n = 2)	0%	85.7% (n = 12)	14.3% (n = 2)	0%
Group n = 7	42.9% (n =3)	57.1% (n = 4)	0%	57.1% (n = 4)	42.9% (n = 3)	0%	100% (n = 7)	0%	0%	100% (n = 7)	0%	0%
Group n = 7	85.7% (n = 6)	14.3% (n = 1)	0	85.7% (n = 6)	14.3% (n = 1)	0%	85.7% (n = 6)	14.3% (n = 1)	0%	100% (n = 7)	0%	0%
	$X^2 = 13.652$ p = 0.003*			$X^2 = 9.563$ p = 0.049*			$X^2 = 3.893$ p = 0.420			$X^2 = 1.400$ p = 0.481		

p<0.05

Table 2: The comparision of the groups in the aspect of focal bleedings in the evaluated tissues

	Periodontal ligament			Alveaolar bone			Gingiva			Pulp		
Groups	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe	No	Mild	Severe
Group n = 14	57.1% (n = 8)	35.7% (n = 5)	7.1% (n = 1)	85.7% (n = 12)	14.3% (n = 2)	0%	100% (n = 14)	0%	0%	76.8% (n = 11)	7.1% (n = 1)	14.3% (n = 2)
Group n = 7	100% (n = 7)	0%	0%	100%	0%	0%	100% (n = 7)	0%	0%	42.9% (n = 3)	0%	57.1% (n = 4)
Group n = 7	100% (n = 7)	0%	0%	85.7% (n = 6)	0%	14.3% (n = 1)	100%	0%	0%	100% (n = 7)	0%	0%
	$X^2 = 6.507$ p = 0.074			$X^2 = 1.400$ p = 0.481			No statstics			$X^2 = 7.330$ p = 0.042		

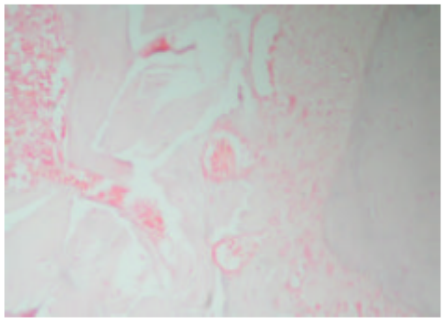

Fig. 2: An example of alveolar bone in exposure group (HE×200)

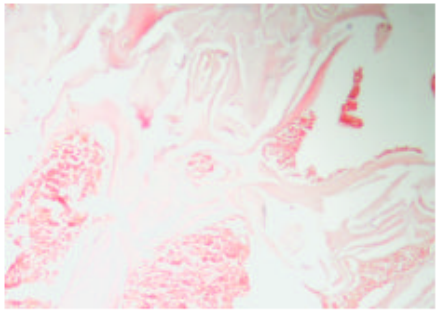

Fig. 3: An example of alveolar bone in sham group (HE×100)

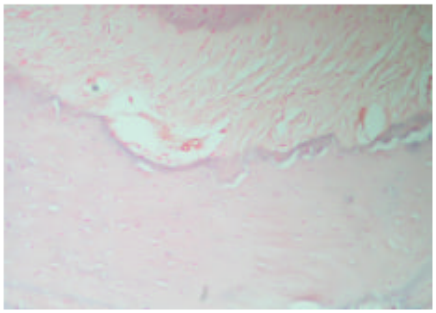

Fig. 4: An example of pulp and periodontal ligament in control group (HE×200)

($p>0.05$), in the case of periodontal ligament and alveolar bone there were statistically significant differences among the groups of control and sham, control and experimental ($p<0.05$).

In the case of periodontal ligament: In the experimental group in 8 individual mild, in 1 individual severe and in 5 individual no vasodilatation was determined.

In the sham group in 4 individual mild and 3 individual no changes were determined.

In the control group in 1 individual mild and in 6 individual no vasodilatation was determined.

In the case of alveolar bone: In the experimental group in 4 individual mild (Fig. 2), in 1 individual severe and in 9 individual no vasodilatation was determined.

In sham groups in 3 individuals severe (Fig. 3) and in 4 individuals no vasodilatation was determined.

In the control group in 1 individual mild and in 6 individual no vasodilatation (Fig. 4) was established.

In the aspect of focal bleedings, although there were differences in the number of positive individual numbers there was no statistical differences among the groups ($p>0.05$).

DISCUSSION

The interaction of RF emitted from mobile phones is still a popular topic, which has been continuing. However, data related to this interaction from scientific articles are controversial. We performed this preliminary observation, which is to investigate periodontal tissues and teeth in rats after exposure emitted from mobile phone.

This research is performed on rats because our aim was to investigate the teeth and surrounding soft and hard tissues histopathologically. By this way we would be able to evaluate the structural and cellular changes in teeth and periodontium. 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.

Long-term animal exposure studies are difficult to accomplish and expensive. Ideally, constant environmental conditions should be maintained throught the experimental period, the handling of test animals should be rigidly controlled and standart operating procedures should be developed and followed. In our study the same standart were maintained.

Most studies of exposure to RF radiation have been of <1 year's duration (Spalding *et al*., 1971; Toler *et al*., 1988; Yilmaz *et al*., 2008). Also, in our study the period was planned as 10 months.

As it is known, GSM phones generally work at three bands, which are 900, 1,800 and 1,900 MHz in the World. In this study, we investigated effects of 900 MHz GSM mobile phone exposure on periodontal tissues and teeth in rat that are sensitive to RF radiation. Because, the use of mobile phones operating i n the 900 MHz frequency band is very widespread and ever increasing (Maes, 2001).

In this study, all structures constituting periodontium and teeth were evaluated in histologic sections. Due to no

changes in cement, dentin and enamel there was no need to show their data in tables.

As we mentioned above, scientific literature in this field is no exist or inadequate. Thus, it is too difficult to discuss the results of this study with others. In addition, we did not find any articles related to interaction of radiation emitted from mobile phones and periodontal tissues and teeth discuss directly. However, we can discuss the results of this study with some articles indirectly.

Some of these studies have measured cancer incidence. In a large epidemiological study, which followed cancer morbidity in the whole population of military career personnel in Poland during a 15-year period (1971-1985), reported that leukemia and brain tumors are significantly higher in RF-exposed personnel compared with unexposed ones (Szmigielski *et al*., 1982). In a case control study, Hardell *et al.* (2002) suggested that the ipsilateral use of an analog cellular phone yielded a significantly increased risk for malignant brain tumors. Hardell *et al.* (2003) also showed an increased risk for brain tumors among users of analog cellular telephones in studies in recent years. No promoting effects of cell phone RFs were observed in a rat liver carcinogenesis model (Imaida *et al*., 1998a, b). In addition, some studies reported that RF radiation did not induce or promote Central Nervous System (CNS) and brain tumors (Salford *et al*., 1993; Adey *et al*., 1999; Higashikubo *et al*., 1999; Adey, 2000; Zook and Simmens, 2001). However, Valberg (1997) reviewed that there are studies arguing that the increased cancer risk reported in connection with exposure is due to various experimental errors and is not the result of the RF radiation. Thus, the question whether exposure to RF radiation leads to cancer, although of grave concern for the general public, still remains a puzzling issue which is awaiting the arrival of more modern tools to be resolved (Mashevich *et al*., 2003).

A few studies assessed life span or have assessed multiple end points related to the overall health of animals (Toler *et al*., 1988; Yilmaz *et al*., 2008). Spalding *et al*., (1971); in studies exposed mice to 800 MHz RF radiation for 2 h/day, 5 days/week, for, 35 weeks. The power density was 43 mW cm^{-2}. End pionts included erythrocyte and leuckocyte count, hemoglobin level, hematocrit, activity level, body weight and life span. No significant differences between the RF-radiation-exposed and sham-exposed groups were seen for any of these measures. However; in the study of Prausnitz and Suskind (1962), which is included for its historical interest, mice were exposed to 9270 MHz RF radiation at 100 mW/cm^2 (4.5 min/day, 5days/week, for 59 weeks). The exposure to RF radiation caused the rectal temperatures to increase by 2-5°C. The mice were followed for up to 83 weeks after the start of exposure. The authors described the presence of a leukocyte neoplasm, which they termed leucosis, as well as testicular degeneration, in exposed animals.

By what mechanism can RF fields perturb biological systems? RF fields are non-ionizing radiations (Fact sheet, 1998). Unlike ionizing radiation or ultraviolet light, the photon energy of RF fields is much too low to break chemicel bonds directly. However, RF fields induce electric fields that result in flow of ions and rotation of asymmetric charged molecules (dipoles). This increase in linear and rotational energy is rapidly dissipated by molecular collisions, which generate heat. The field-induced molecular rotation is known as dielectric dispersion and is maximal for a given dipole at a characteristic relaxation frequency. At 900 MHz, the dominant relaxation phenomenon (in which there is a rapid change in the dielectric constant and conductivity of the absorbing tissue) is the δ-dispersion, which results from the relaxation of bound water, amino acids and charged side chains in proteins. Theδ-dispersion and, to a lesser extent, the other relaxation phenomena are responsible for the eventual heating of tissue after absorbsion of RF energy (UNEP/IRPA/WHO, 1993; Kaplan *et al*., 1983).

In our study it can be seen that there was no statistical difference determined among the experimental and sham groups. This result is harmonical with some other studies in which 900 MHz RF radiatio was exposed (Spalding *et al*., 1971; Toler *et al*., 1988; Yilmaz *et al*., 2008). Although, there was no statistical difference determined among the groups of experimental and sham groups, there were numerically more individuals effected of RF radiation in the experimental group. Another important finding of our study is the statistically significant difference among the groups of experimental, sham and control groups in the case of vasodilatation in periodontal ligament and alveolar bone. These findings of our study indicates that there is need to more animal, human being and epidemiologic studies including much more individuals.

CONCLUSION

The case if the mobile phones treath or not the community health is stil not clarified. There is no study in literature about the RF radiations effects on teeth and periodontal tissues as it is the topic of our study. This study is important because it is the first study in this field. The answer of the questionhave mobile phones have effects on teeth and periodontal tissues was investigated in this study. Although, the number of rats whose tissues were effected in the experimental group were numerically

more than the sham group the was so statistical significancy among them. Because of this, the answer of the question have mobile phones have effects on teeth and periodontal tissues couldn't be fully constituted. But we believe that it can be answered by more detailed further studies. And we think that our study may be a guide to those further studies.

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