

Effects of Electromagnetic Radiations from Mobile Phone on Gingiva in the Era of 4g Lte-An *In Vivo* Study in Rabbits

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Abstract

Background: Mobile phones have become an essential part of our life. They emit electromagnetic radiation which is harmful to the body. Studies on various organs/tissues motivated us to examine the effects of electromagnetic radiation on the gingiva as the mobile phone is held close to the oral cavity when in speech mode.

Aim: The present study was aimed to analyze the effect of Electromagnetic Radiation (EMR) induced by cellular phone on the gingiva of the rabbit-an in vivo animal study.

Materials and methods: 15 rabbits were divided into 3 groups, each group comprising of 5 rabbits and housed in standard cages. Group 1 was exposed to a mobile phone for 8 hours/day in speech mode and 16 hours/day in standby mode for 3 months. Group 2 was exposed to the cellular phone for 24 hours continuously in standby mode for 3 months and Group 3 was the control group without any exposure to electromagnetic radiation. After 3 months the gingiva was biopsied under light microscope and electron microscope.

Results: Although there was no statistical difference determined among the experimental and control groups ($p > 0.05$) in terms of structural and cellular changes in the epithelium, connective tissue and blood vessels in the gingiva, samples in group 1 showed presence of more inflammatory cells especially large and small lymphocytes which are characteristic of chronic inflammation.

Conclusion: These findings of the study indicate that there is a need to do more animal, human being and epidemiological studies including much more individuals for a longer duration of time.

Keywords: Mobile phones; Electromagnetic radiation; Gingival disease; Inflammation; Connective tissue

Introduction

We are exposed to an ocean of Electromagnetic Radiation (EMR) produced by various sources such as electrical appliances, power lines, wiring in buildings and other technologies associated with the comfort of modern life. These appliances may even include the dishwasher and microwave oven utilized in the kitchen or the digital wall clock, to the cellular phone which is usually held close to the ears. Thus, exposure to EMR is alarmingly growing and poses a serious health hazard.

Mobile phones emit electromagnetic radiation-when the power is turned on or even in standby mode regardless of whether carried on belts, in pockets or purses, mobile phones, expose areas of the body to harmful radiations. Many studies have linked the exposure of radiation from the handsets and the tower-based antennas carrying the signals to the development of brain tumors, genetic damage, and other exposure-related conditions.

Currently, there are around 2 billion smartphone users worldwide whereas 83% of global internet users prefer their mobile devices to go online. A major bulk of internet usage is detected to be from mobile or smartphones. The advancement in wireless technology holds the key for further expansion of growth of internet to access even

the remotest part of the world especially in the Least Developing Countries (LDCs) [1].

Studies have indicated that mobile phone can have an adverse impact on human health causing cancer, hearing capability, sleeping disorder and blurring vision. Even though all the above adverse effects have not been proved scientifically from a medical perspective but we cannot ignore the possible consequences. It is crucial that in every aspect the mobile phone makes our life easier. Mobile phones emit low levels of radiofrequency in the microwave range while being used. Although high levels of radiofrequency can produce health effects (by heating tissue), exposure to low-level radiofrequency may not produce heating effects and causes no known adverse health effects [2,3].

There is credible scientific evidence that radiofrequency exposures cause changes in cell membrane function, metabolism, and cellular signal communication, as well as activation of protooncogenes and triggering of the production of stress proteins at exposure levels below current regulatory limits. There is also a generation of reactive oxygen species, which cause DNA damage, chromosomal aberrations and nerve cell death [4-7].

Numerous research studies have been conducted over the years on the effect of electromagnetic radiation on various organ/tissues, which motivated us to analyze the effect of electromagnetic radiation on gingiva as the mobile phone is held close to the oral cavity when in

speech mode. According to literature till date, there is very minimal or no existing document on the effect of cellular phone induced radiation on the gingiva. Thus, the aim of the study was to evaluate and compare the effects of cellular phone radiation on gingiva in the experimental group of rabbits in speech mode and standby mode with a control group of rabbits without any exposure.

Materials and Methods

The study was approved by Institutional Animal Ethics Committee (IAEC) held on 07/01/2015 at Rajarajeswari Medical College and Hospital, Bangalore, with the study protocol No: IAEC-RRMCH 09/2015 and it was resolved to use 15 rabbits for the research project. The study was also approved by the ethical committee of Rajarajeswari Dental college and hospital, Bangalore with the reference No: RRDC and H/270/2013-14 on 27/11/2013.

Rabbits housed at animal house of Rajarajeswari Medical College and Hospital, Bangalore were included in the study based on the following inclusion and exclusion criteria. Inclusion criteria were-(i) Male rabbits, (ii) Rabbits in the age group of 6-8 months and (iii) Healthy rabbits without a history of any disease. Exclusion criteria were-(i) Female rabbits, (ii) Diseased rabbits and (iii) History of previous gum or periodontal disease.

A total of 15 rabbits were included and divided into 3 groups, each group comprising of 5 rabbits and housed in standard cages (Figure 1). Group 1-was exposed to a mobile phone for 8 hours/day in speech mode and 16 hours/day in standby mode for 3 months. Their gingiva was biopsied for examination under light microscope and electron microscope. Group 2-was exposed to the cellular phone for 24 hours continuously in standby mode for 3 months and their gingiva was biopsied for examination under light microscope and electron microscope.

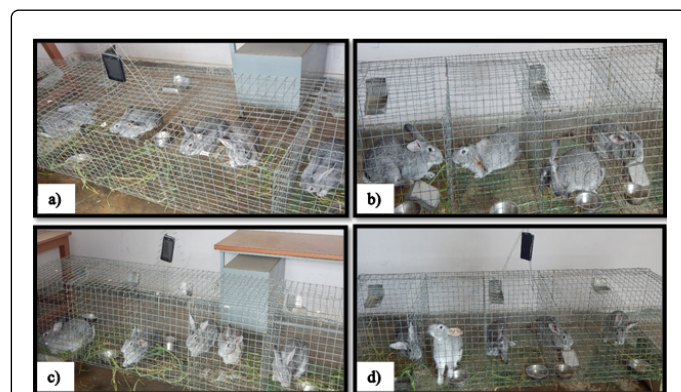


Figure 1: a) Rabbit housed in the cage for the study; b) Rabbits in the control group; c) Rabbits in the test group 1; d) Rabbits in the test group 2.

Group 3-was the control group without any exposure to electromagnetic radiation and their gingiva was biopsied under a light microscope and transmission electron microscope (TECNAI 200kV TEM). From each group, two excisional biopsy samples were collected at baseline and after 3 months, for examination under light and electron microscope.

Subjects and animal care

Fifteen (15) healthy Soviet Chinchilla adult male rabbits of age 8-12 months with initial average weight 2800 ± 1000 grams were obtained from the animal house facility of Rajarajeswari Medical college and hospital, Bangalore, and the rabbits were kept in separate (different) three rooms in standard cages of $300\text{cm} \times 60\text{cm} \times 60\text{cm}$ and each rabbit individually in the cage size of $60\text{cm} \times 60\text{cm} \times 60\text{cm}$ and fed with standard pelleted food and water. All the rabbits were monitored in a standard laboratory environment on a 12 hours light and dark cycle and the required temperature and humidity was maintained. All the vital signs like (a) pulse, (b) blood pressure, (c) respiratory rate, and (d) temperature, were recorded by a veterinarian every fortnight.

Description of mobile

In the present study, Huawei Y530[®] (China) mobile phone was used as a 900-MHz continuous wave electromagnetic energy generator, which has the highest specific absorption rate (SAR), 1.15 W/kg [Head: 0.45 W/kg, Body: 0.93 W/kg, Product Specific Use: 0.93 W/kg, Simultaneous Transmission: 1.15 W/kg in the market. Mobile phones were fixed in a hanging position 12 cm above the cages. Mobile phones were left on charge for 24 hours, and 8 hours/day in speech mode in test group 1 and 24 hours/day in standby mode in test group 2 (Figure 2).



Figure 2: Mobile phone used in the study-Huawei Ascend Y530.

Gingival sample collection

Before the beginning of the study, the gingival samples were biopsied and studied for the confirmation of gingival health and non-inflammatory status of the gingiva. The rabbits were sedated with an intramuscular injection of 1ml of Xylazine (Xylaxin-30 ml vial) and 0.5 ml of Lignocaine with 2% adrenaline 1in 80,000 (Xicaine) which was injected as local infiltration at the gingival site. Two gingival tissue samples were obtained from the upper anterior and lower anterior region in the size of $2\text{mm} \times 2\text{mm}$. and fixed in 10% formalin for light microscopic examination and the other sample was fixed in glutaraldehyde (2.5% solution in phosphate buffered saline) and

prepared for electron microscopic examination. After 3 months, the tissue samples were obtained and processed in a similar manner for light microscopic examination and electron microscopic examination (Figure 3).

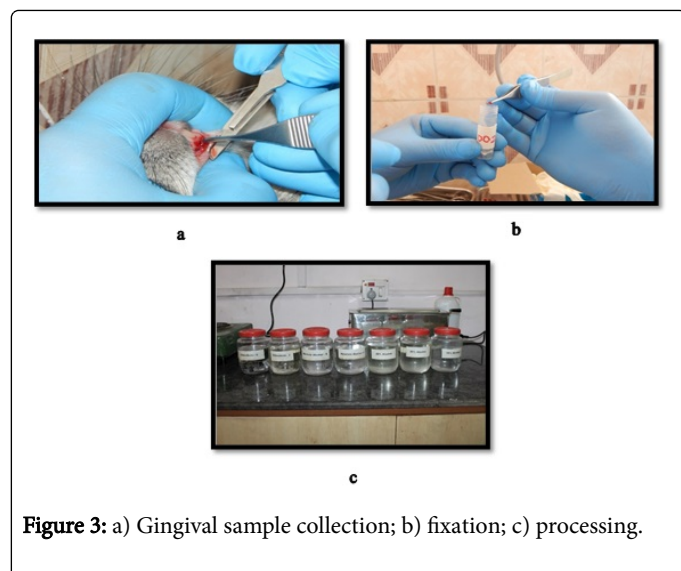


Figure 3: a) Gingival sample collection; b) fixation; c) processing.

Preparation of tissue specimen for simple microscopic examination

The tissue samples were obtained and fixed in 10% formalin for further processing. For processing, the tissue samples were removed from formalin bottles and placed in filter paper and put in small capsules and stored in 10% buffered formalin (4% formaldehyde in phosphate buffered saline) solution overnight. The capsules were removed from 10% buffered formalin solution and washed in running water for 10 minutes. After washing for 10 minutes the specimen capsules were placed in 70% alcohol for 60minutes. After 60 minutes, the specimen capsules were removed and placed in 80% alcohol for 60minutes. After 60 minutes, the specimen capsules were removed and placed in 90% alcohol for 60minutes. After 60 minutes, the specimen capsules were removed and placed in absolute alcohol-I for 60minutes. After 60 minutes, the specimen capsules were removed and placed in absolute alcohol-II for 60minutes.

After 60 minutes, the specimen capsules were removed and placed in chloroform-I for 60minutes. After 60 minutes, the specimen capsules were removed and placed in chloroform-II for 60minutes. After 60 minutes, the specimen capsules were removed and placed in paraffin wax bath overnight (Figure 3).

Embedding

After the tissues were dehydrated, cleared, and infiltrated with the embedding material, they were ready for external embedding. During this process, the tissue samples were placed into molds along with liquid embedding material (paraffin wax) which was then hardened. This was achieved by cooling of paraffin wax. The hardened blocks containing the tissue samples were ready to be sectioned.

Sectioning

For light microscopy, a steel knife mounted in a microtome was used to cut the tissue sections into 4-micrometer-thick tissue sections which were mounted on a glass microscope slide.

Staining

Staining is employed to give both contrasts to the tissue as well as highlighting particular features of interest. Hematoxylin and Eosin (H and E stain) were used for staining the glass microscope slide and the slides were ready for light microscopical examination.

Preparation of tissue specimen for Transmission Electron Microscopic examination

The gingival tissue samples obtained from the upper anterior and lower anterior region in the size of 2mm × 2mm. and fixed in glutaraldehyde (2.5% solution in phosphate buffered saline) was sent to All India Institute of Medical Sciences (AIIMS) for preparation of slides and transmission electron microscopic examination.

Statistical analysis

The Statistical software namely SPSS 15.0, Stata 8.0, Medical 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables etc. The values obtained were subjected to statistical analysis. The test of significance was applied Fisher's exact test (two-tailed) was applied between the groups to know the overall difference among the groups.

Results

The statistical analysis of data revealed that there was no statistical difference between the three groups ($p=1.000$ between groups 1 and 2; $p=0.44$ between groups 2 and 3 and $p=0.44$ between groups 1 and 3). The overall difference among groups was $p=0.251$ which were also not statistically significant.

Among all the groups, two samples had inflammation in the gingival tissues (40%) in group 1, two samples had mild inflammation the gingival tissues (40%) in group 2 and none of the samples had any inflammatory changes in group 3. In all the groups, light microscopic examination of the gingival tissues revealed para keratinized stratified squamous epithelium of varying thickness covering the underlying connective tissue.

The connective tissue in group 1 showed numerous bundles of collagen fibers with few inflammatory cells and minimal dilatation of blood vessels (Figure 4a). The collagen fiber bundles and inflammatory cells were lesser in group 2 when compared to group 1 (Figure 4b). In group 3, the connective tissue showed loosely arranged fibrous tissues devoid of inflammatory cells with numerous bundles of collagen fibers and normal undilated blood vessels (Figure 4c).

The transmission electron microscopic examination of the gingival tissues revealed para keratinized stratified squamous epithelium of varying thickness covering the underlying connective tissue in group 1 (Figure 4d). Similar keratinization was observed in groups 2 and 3. The connective tissue revealed fewer inflammatory cells and fewer minimally dilated blood vessels in group 2 (Figure 4e) as compared to group 1. The connective tissue showed no inflammatory cells with the normal undilated lumen of blood vessels and a normal epithelium-connective tissue interface in group 3 (Figure 4f).

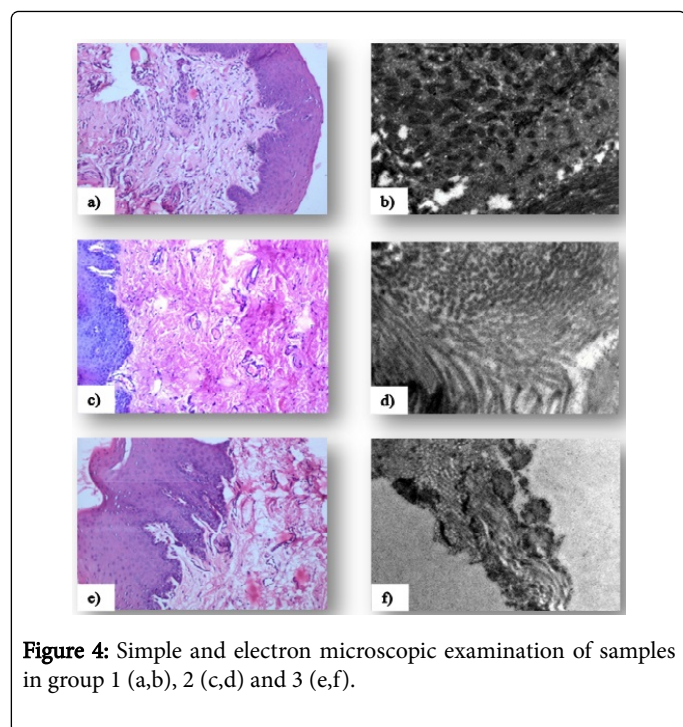


Figure 4: Simple and electron microscopic examination of samples in group 1 (a,b), 2 (c,d) and 3 (e,f).

Although the results between the three groups were statistically not significant, samples in group 1 showed the presence of more inflammatory cells especially large and small lymphocytes which are characteristic of chronic inflammation when compared to groups 2 and 3. The blood vessels in group 1 showed increased dilatation of blood vessels with enlarged lumen when compared to groups 2 and 3 (Figure 5).

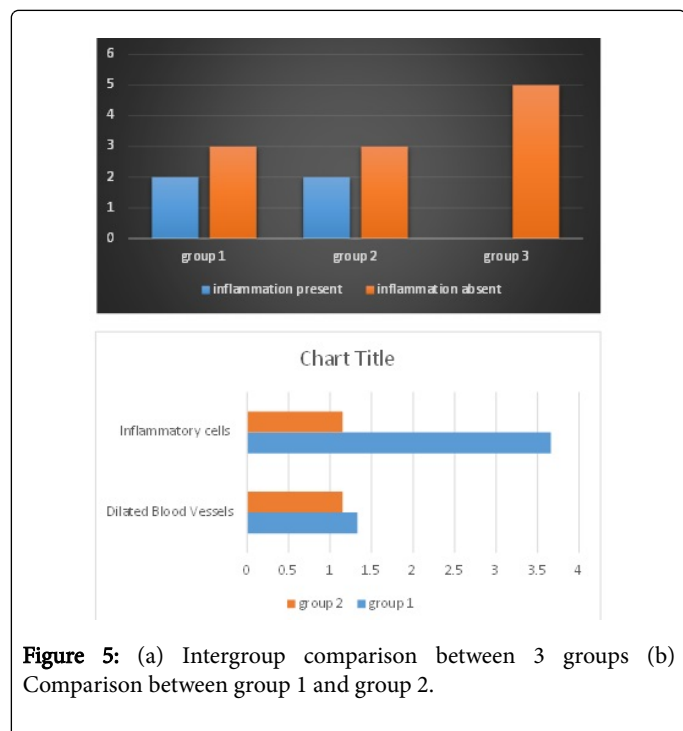


Figure 5: (a) Intergroup comparison between 3 groups (b) Comparison between group 1 and group 2.

Discussion

The electromagnetic spectrum contains an array of electromagnetic waves increasing in frequency from the extremely low frequency and very low frequency. A common concern today is that mobile phone antennas radiate near a person's head. Exposure to electromagnetic fields has been linked to a variety of adverse health outcomes [8]. Scientific evidence has shown that exposure to radiofrequency can result in changes in cell membrane function, metabolism, and intercellular communication. It can also lead to the activation of proto-oncogenes and trigger the release of stress proteins at exposure levels below current regulatory limits. There are studies in the literature which have proven the production of reactive oxygen species, subsequent DNA damage, chromosomal aberrations and nerve cell death upon exposure to such radiations [9].

As a part of the protocol, the release of mobile phones into the market should be preceded by their compliance with the requirements of European directives, i.e., the limits for the amount of power absorbed in the human body should not be exceeded. The limit for mobile phone use is the Specific Absorption Rate (SAR) of 2 W/kg for the human head. Maximum local SAR values averaged over 10 gram of tissue range typically between 0.2 and 1.5 W/kg. These values also depend upon the type of mobile phone.

In the currently available GSM phones, care is taken to keep the emitted power lower than the maximum power, to decrease the exposure, at a lower power control and discontinuous transmission mode. There is no exposure from a mobile phone which is in power off mode. The standby mode has been shown to cause typically much lower exposure compared to mobile phones that are in operation with utmost power [10].

The Council Recommendation of 12 July 1999 on the limitation of exposure of the general public to electromagnetic fields (0 Hz to 300 GHz) has fixed basic restrictions and reference levels to Electromagnetic Fields (EMFs). These restrictions and reference levels are based on the guidelines published by the International Commission on Non-Ionising Radiation Protection (ICNIRP). The ICNIRP guidelines had been endorsed by the Scientific Steering Committee (SSC) in its opinion on the health effects of EMFs of 25-26 June 1998 [11].

Numerous scientific publications and reviews on the possible health effects of EMF (focusing mostly on mobile telephones) have become available since the CSTE opinion of 2001, for example the 2002 Dutch report [12], the 2003 AGNIR report [13] and the 2004 British National Radiological Protection Board (NRPB) report on "Mobile phones and health", which is the most recent of them. It was concluded from the NRPB report that no hard evidence is currently available to state that the health of the public is being adversely affected by mobile phone technologies but uncertainties remain and a continued precautionary approach has been suggested until clarification of the situation [14].

Data related to the interaction of radiofrequency emitted from mobile phones is controversial. We performed this preliminary observation which is to investigate the gingival tissues in rabbits after exposure emitted from mobile phones. This research was performed on rabbits because our aim was to investigate the gingival tissues histopathologically. In this way, we were able to evaluate the structural and cellular changes in the epithelium, connective tissue, and blood vessels. All animal procedures were in agreement with the principles of

laboratory animal care and the rules of scientific and ethics committee of Rajarajeswari Dental College and Hospital.

Animal exposure studies with a long-term follow up are difficult to accomplish and expensive. Ideally, constant environmental conditions should be maintained throughout the experimental period. A rigid control and standard operating procedures should be developed and followed for the handling of test animals. In this study, the same standards were followed. Most studies of exposure to radiofrequency radiation have been of less than one-year duration (Spalding et al.; Toler et al.; and Yilmaz et al.) [15-17]. In the present study also the investigation was carried out for a period of 3 months.

As it is known that GSM phones generally work at 3 bands, which are 900, 1800 and 1900 MHz in the world. In this study, effects of 900 MHz GSM mobile phone exposure on gingival tissues in rabbits that are sensitive to radiofrequency radiation were investigated. Because the use of mobile phones operating in the 900 MHz frequency band is very widespread and ever-increasing [18]. In this study gingival structures like cells of the epithelium, connective tissue and blood vessels were evaluated in histological sections. As mentioned earlier, scientific literature in this field is inadequate. Moreover, there are no direct articles related to the interaction of radiation emitted from mobile phones and gingival tissues of rabbits.

Studies conducted in the past have shown contrasting results regarding the effects of mobile phone radiation on specific organs. Various *in-vitro* studies have been conducted in experimental systems to investigate the possible carcinogenicity of radiofrequency field exposure. The results of those experiments were essentially negative. An interesting exception is that of Repacholi et al. [19], who had induced a two-fold increase in lymphoma incidence in a strain of lymphoma-prone transgenic mice (E μ -Pim1) following exposure to 900 MHz RF fields with a signal similar to the GSM modulation. In contrast, Utteridge et al. [20] failed to confirm the results of the Repacholi et al. study. Utteridge and co-workers found that exposure to radiofrequency fields had no statistically significant effects on the incidence of lymphoma.

Several other studies investigated the effect of exposure to radiofrequency fields on the development of tumors induced by chemical carcinogens, X-rays or UV radiation [21-25]. No statistically significant increase of tumor incidence has been reported in any of these studies [26]. Twelve research groups in seven European countries performed the REFLEX study to investigate the basic mechanisms induced by EMF using toxicological and molecular biological technologies at cellular and sub-cellular levels *in vitro*. The investigators [27] reported that exposed DNA strand broke in human diploid fibroblasts and cultured rat granulosa cells. Participants of the REFLEX-study reported no effects of radiofrequency fields on cell cycle, cell proliferation, cell differentiation, apoptosis induction, DNA synthesis, and immune cell functionality [27].

In our study, it was revealed that there was no statistical difference determined between the experimental and control groups. This result is harmonical with some other studies in which 900 MHz radiofrequency was exposed [28-30]. Samples in group 1 showed the presence of more inflammatory cells especially large and small lymphocytes which are characteristic of chronic inflammation and the connective tissue showed increased dilatation of blood vessels with enlarged lumen when compared to the other groups. There were numerically more individuals affected of radiofrequency radiation in the experimental groups. These findings of our study indicate that

there is a need for more animal, human being and epidemiological studies including much more individuals (sample size) for a longer duration of time.

Conclusion

Within the limitations of the present study, it can be concluded that there is no effect of cellular phone radiation on the gingival tissues in rabbits. Although short-term animal studies are considered less relevant they can provide important contributions to understanding the mechanisms of electromagnetic effects. The findings of our study indicate that there is a need to do more animal, human being and epidemiological studies including much more individuals for a longer duration of time.

Conflict of Interest

No conflicts of interest were expressed

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