

Occurrence of Micronuclei in Exfoliated Buccal Mucosal Cells in Mobile Phone Users: A Case-Control Study

Abstract

Introduction: The use of mobile phones has increased enormously all over the world especially among young people. This technology is based upon electromagnetic radiation in the microwave frequency range [radiofrequency (RF) waves and microwaves]. The radiation frequency and modulation standards vary in the range of 300–2100 MHz, depending on the region in the world. **Aim and Objectives:** To detect the presence of micronuclei in exfoliated buccal mucosal cells of mobile phone users as well as to carry out a quantitative analysis of micronuclei in exfoliated buccal mucosal cells of mobile phone users. **Material and Methods:** A cross-sectional study was carried out in 100 individuals, out of which 50 were included under the test group and 50 under the control group. Buccal mucosal smears were obtained from each subject. Staining was done using papanicolaou (PAP) stain and the slides were examined for the presence of micronuclei. **Results:** The mean duration of mobile phone usage in years was 5.32 years for the exposed and 2.42 years for the control. Mean duration of mobile phone usage per day in the test group was 94.8 min and 12.4 min in the control group. The frequency of micronucleated cells (MNC) in test and control groups ranged from 0 to 16 and 0 to 2 respectively. The data obtained were statistically analyzed using Student t-test and significant results were obtained at 0.0001 level. **Conclusion:** Our study concluded that there is an increased frequency of micronuclei in mobile phone users which is related to carcinogenesis.

Keywords: Electromagnetic radiation, micronuclei, mobile phone

Introduction

The use of handheld cellular mobile telephones is enormously increasing day by day. India ranks fourth, behind China, USA, and Russia, which have approximately 450, 233, 160, and 156.3 million subscribers respectively.^[1] Mobile phones are now an integral part of modern life. It operates within the radiofrequency range of 30KHZ–300MHZ of the electromagnetic spectrum, the microwaves. Some adverse effects reported from exposure to microwaves include physiological, neurological changes, carcinomas, and other biological damage through heating effects.^[2] Regeneration is dependent on the number and division rate of the proliferating basal cells and their genomic stability and propensity for cell death.^[3] Keeping in view the above facts, the aim of the present study was to investigate the presence of micronuclei in exfoliated buccal mucosal cells of mobile phone (smartphone) users as

smartphone emits more radiation compared to regular phones.

Materials and Methods

This study was carried out in 100 Indian individuals. Study group comprised of 50 individuals who use mobile phones more than 2–3 hours per day for a minimum of 4 years duration. Control group comprised of 50 patients with the usage of mobile phones less than half an hour per day. The inclusion criteria include both study and control groups belonging to the same age group of 19–45 years with apparently normal oral mucosa. The exclusion criteria include Smokers and tobacco users, individuals with oral lesions, and systemic disorders like diabetes and hypertension. Case history and personal information were assessed, including age, gender, smoking and alcohol consumption, exposure to chemical carcinogens or radiation and dietary habits. A questionnaire addressing the duration of daily mobile phone use, overall period of exposure, preferential side, and the use

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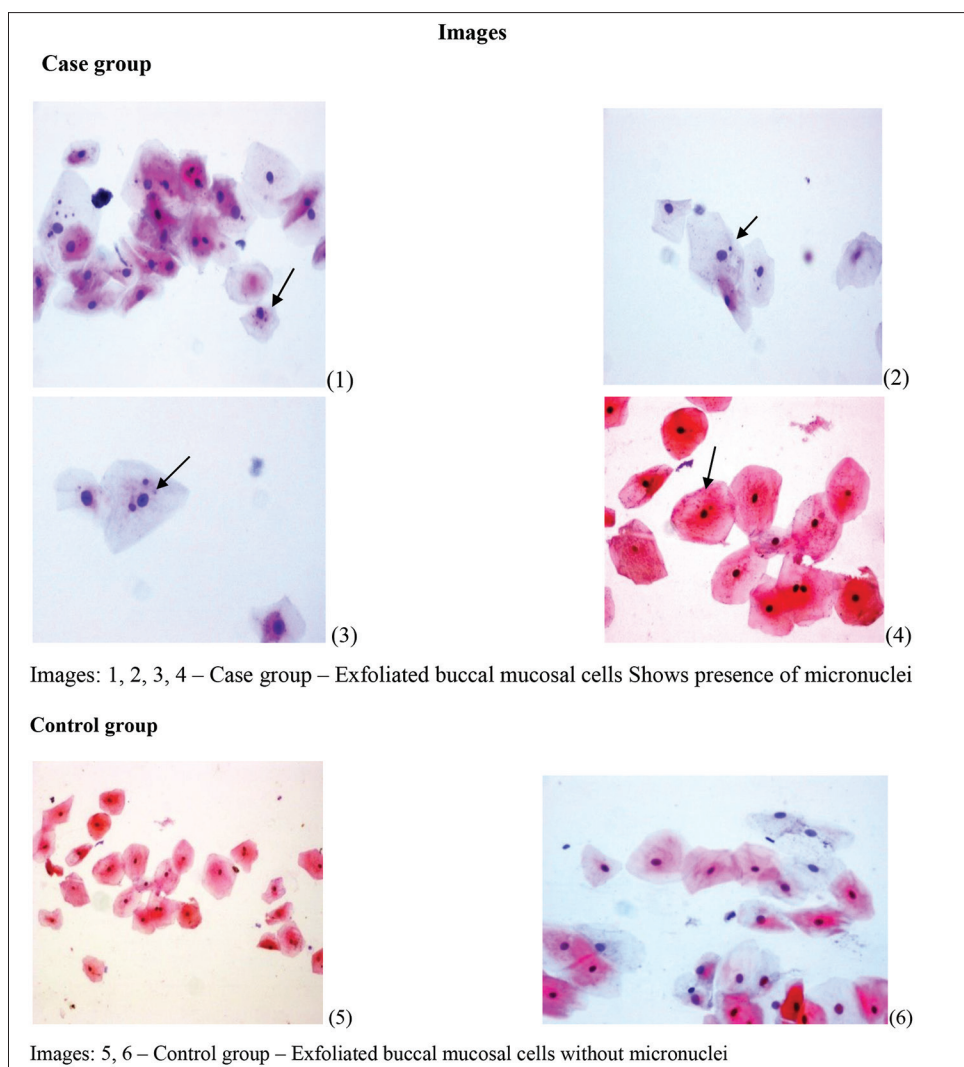


Figure 1: Exfoliated buccal mucosal cells in case and control group

of headsets was completed. The study was approved by the Ethical committee (MRADC & H/ESIRB/29/2011-12) of MR Ambedkar Dental College & Hospital, Bengaluru, Karnataka, India. All subjects were fully informed about the purpose of the study and their consent was obtained.

Prior to the collection of cells, the mouth was rinsed with water to remove saliva, food particles, and any other debris. Buccal mucosal smear was taken from each subject by using a wooden spatula [Figure 1]. The smear was fixed with Biofix spray and stained using Rapid PAP (3 minutes Papanicolaou stain). All the slides were observed under a light microscope using magnification ($\times 100$) for screening and magnification ($\times 400$) for counting of micronuclei. The most commonly used was the zig-zag method for screening of the slides. Cells with intact nuclei and cell boundaries were counted. A total of 1000 cells were counted in the smear from the predominant buccal mucosa of each patient for the presence and number of micronucleated cells (MNC) and the number of micronuclei (MN) in

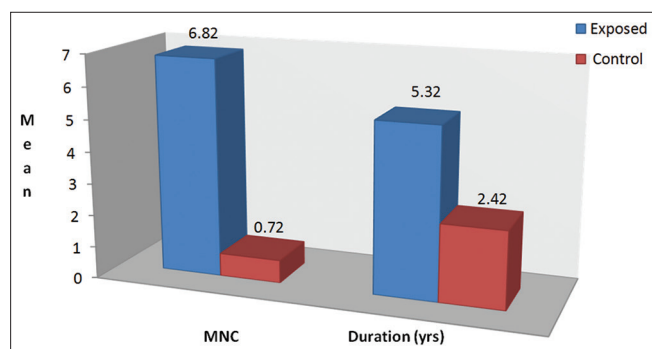
each cell. The percentage frequencies of MNC and MN were recorded. The charge-coupled device (CCD) camera should be used to capture the pictures. The events can be studied in the buccal mucosa, which is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study subjects.^[4] The buccal micronucleus cytome (BMNcyt) assay is a minimal invasive method for studying DNA damage, chromosomal instability, cell death, and the regenerative potential of human buccal mucosal tissue.^[5,6]

Statistical analysis

Data were collected and statistically analyzed using Student's *t-test* and results were expressed in mean $\pm$ standard error of mean. The results were significant statistically with $P < 0.0001$.

Results

The mean age of the test and control group was 23.06 and 26.86 respectively. Mean duration of mobile phone usage



Graph 1: Mean of comparison between micronuclei and duration of mobile phone users in exposed and control group

per day in the test group was 94.8 min and 12.4 min in the control group [Table 1]. The frequency of MNC in test and control groups ranged from 0 to 16 and 0 to 2 respectively. The mean percentage frequencies of MNC in the test group and controls are shown in Table 2. The mean number of cells with micronuclei in the exposed group and the control group was 6.8 and 0.7. The mean duration (year) of mobile phone users in the exposed and control group was 5.32 years and 2.42 years [Graph 1]. The data obtained were statistically analyzed using Student's *t*-test and significant results were obtained at ($P = 0.0001$) level.

Discussion

The connection between mobile phone usage and cancer is a concern for the world population. Micronuclei are one of such biomarkers that are cytoplasmic chromatin masses with the appearance of small nuclei that arise from lagging chromosomes at anaphase or from a centric chromosome fragments. DNA damage (micronuclei and / or nuclear buds), cytokinetic defects (binucleated cells), proliferative potential (frequency of basal cells), and / or cell death (condensed chromatin, karyorrhexis, pyknotic and karyolytic cells) have been related to a high risk of cancer, aging neurovegetative disorders and to the carcinogenic process with increased genetic instability. Alterations of genetic information in somatic cells are the key events in the process of carcinogenesis.^[7]

Apart from the well-documented and widely accepted heating effect of radiofrequency at higher radiation power settings, the controversy over whether such radiation exerts effects upon biological systems has been going on for a long time and is almost as old as the widespread use of mobile phone technology.^[5] International Agency for Research on Cancer (IARC) has classified low-frequency electromagnetic field as a possible carcinogen. Low electromagnetic field may promote DNA damage and hence may be genotoxic. Micronucleus assay is a multi endpoint test of genotoxic responses to clasto genesis & is extensively used as a biomarker of genomic instability.^[1] Few studies have been based on exfoliated cells. We chose exfoliated oral mucosal cells because the oral cavity is

Table 1: General characteristics of study group and control group

Variables	Exposed Group	Control Group	Total
Average age (in years)	23.06	26.86	24.97
Duration (in years)	5.32	2.42	-
Duration (in min)	94.8	12.4	-
Total	50	50	100

Table 2: Comparison of Mean value and Standard Deviation of micronuclei in exposed & control group

	Mean no. cells with micro nuclei±(SD)	SE	Range
Micro nucleated cells (MNC)			
Exposed Group	6.82±0.714	5.05	0-16
Control Group	0.72±0.099	0.70	0-2

$P=0.0001$ (Significant)

located within the area of mobile phone radiation, and epithelial tissue is the target tissue for carcinogenetic damage.^[5] The test used is rapid and easy to use, as oral mucosal cells do not need to be cultivated like different types of blood cells—especially lymphocytes, which must be stimulated to undergo mitosis. Epithelial cells do not need to be stimulated. Micronuclei in exfoliated cells reflect genotoxic events that occurred in the dividing basal layer 1–3 weeks earlier.^[1] Our study recorded statistically significant changes in micronucleus frequency according to subject age and duration of mobile phone use. This is in agreement with most of the studies that have used the oral mucosa micronucleus test (Gandhi and Singh, 2005). The aim of the study was to detect any cytogenetic damage in mobile phone users by analyzing short term peripheral lymphocytes cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.^[2] In 2008, Yadav and Sharma studied exfoliated oral mucosal cells in 109 subjects (85 users and 24 controls), applying the BMNcyt assay. A slight increase in frequencies was observed, but the difference failed to reach statistical significance. An important finding from this study is that the duration of exposure, MN and total micronuclei (TMN) frequency shows a positive correlation in initial years of exposure, i.e., 0–1, 1–2, 2–3, 3–4 years but a slight decrease in the frequency in subjects exposed to more than 4 years. It may be possible that some DNA repair mechanism gets activated, when it is exposed to prolonged use of low-frequency electromagnetic radiation *in vivo* conditions, which worked to protect DNA.^[1] In 2010, Hintzsche and Stopper investigated the effect of mobile phone usage on genomic instability in human oral mucosa cells. A total of 131 individuals (13 non-users and 85 users for 3 hours or

less a week, and 33 users for more than 3 hours a week) were evaluated for the frequency of MN and other nuclear aberrations, applying the MN test. No significant difference was observed.^[5] In 2007, L Popova studies suggested that panoramic X-ray radiography does not induce a detectable increase in the number of micronuclei in buccal epithelial cells. In conclusion, radiation exposure from panoramic radiography does not induce a statistically significant increase in the number of micronuclei in the target tissue of buccal epithelium analyzed.

The frequency of buccal cell micronuclei was not found to be increased following radiographic 'examination compared with the frequency prior to radiation exposure. Low radiation absorbed doses could be a reason for the negative results. Age, but not gender of the donors correlates significantly with the initial micronucleus frequency.^[3] Several studies involving human fibroblasts, lymphocytes, and erythrocytes (d'Ambrosio *et al.*, 2002; Vijayalaxmi *et al.*, 2003; Zeni *et al.*, 2003, 2008; Ferreira *et al.*, 2006; Scarfi *et al.*, 2006; Verschaeve *et al.*, 2006; Juutilainen *et al.*, 2007; Ziemann *et al.*, 2009; Yildirim *et al.*, 2010; Kesari *et al.*, 2011) have described the effects of exposure to RF-electromagnetic fields at frequencies used for communication with mobile phones. These studies, mentioned above, evaluated the presence of MN in cultivated blood cells by comet assay, MN test, and / or BMNcyt assay.^[7]

Conclusion

Our study concluded that there is an increased frequency of micronuclei in mobile phone users which is related to genetic damage. It may be possible that some DNA

repair mechanism gets activated, when it is exposed to prolonged use of low-frequency EMR *in vivo* conditions, which worked to protect DNA. It is conjectured that some clandestine mechanism helps DNA to develop some sort of adaptability; however, when it is in continuous exposure of week genotoxic agents such as low-frequency EMR, it is a matter of further investigation.

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Conflicts of interest

There are no conflicts of interest.

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