

Evaluation of Micronuclei in Buccal Smears of Mobile Phone Users: A Comparative Study

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ABSTRACT

Background: The mobile uses have been increased drastically, creating increased health concern about radiation effects emitted from mobile phone. Hence, this study aimed to evaluate the micronuclei (MN) frequency in exfoliated oral mucosal cells in high and low mobile users. **Materials and Methods:** The total of 50 subjects was divided into two major groups: low mobile phone users and high mobile phone users. Further, subjects who use mobile phone for more than 10 h a week were considered high talk time users and <4–5 h a week was low talk time users. The buccal mucosa cells are extracted by scraping the oral cavity with a wooden spatula. MN was evaluated in 1000 cells per individual at the microscope.

Results: There was a significantly increase in MN count in high mobile users compared to low mobile users. There was a highly significant difference in the mean MN count of participants using code division multiple access than global system for mobiles mobile phones. The MN mean count was found to be significantly increased in nonheadphone users in comparison to headphone users. Furthermore, the MN count on the frequently used side of mobile phone was found to be statistically significantly elevated compared to the opposite side.

Conclusions: Mobile phone radiation, even in the permissible range when used for a longer duration, can cause significant genotoxicity. The genotoxicity is noted when the mobile phone is used more frequently on the same side, which may be due to more amount of radiation and increase in the temperature. Headphone usage reduces the genotoxicity of mobile phone radiation to some extent.

KEYWORDS: Buccal mucosa, micronuclei, mobile phone, radiation

INTRODUCTION

In this emerging era of mobile phones, more than 3 billion people are using mobile phones in the world.^[1] India has world's second-largest population of mobile phone users,^[2] having the "teledensity" of 80%.^[3] One of the concerns of cell phone users is the prolonged exposure to harmful and potentially carcinogenic waves through talking.^[4] With the widespread use of mobile phones, even a small increase in its carcinogenic risk can have a significant impact on public health.^[1] Mobile phones use microwave radiation in the carrier frequency range of 900–1800 MHz.^[5] The mobile phone radiates an average power of 0.2–0.6 W, 40% of which is absorbed

in hand and the head. It may be regarded as a quite "powerful radio transmitter." Its emission is 10,000 times stronger than that reaching the head of a user standing within 30 m range of the relay transponder.^[6]

According to the International Agency for Research on Cancer, the mobile radiations are classified as Group 2B,

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possibly carcinogenic radiations, i.e. there “could be some risk” of carcinogenicity.^[7] This leads to the concern of possible adverse health effects on mobile phone users from exposure to electromagnetic radiation. The possible oral cell changes, according to Fenech^[8] include DNA damage such as micronuclei (MN) or nuclear buds, cytogenetic defects such as binucleated cells, proliferative potential, and/or cell death have been related to a high risk of cancer, aging, and to the carcinogenic process.^[9] Usually, a cancer diagnosis is made in advanced stages because there are no early obvious clinical signs.^[10,11] However, nuclear alterations occur strongly in the early stages of cancer. These changes in buccal cells were introduced for the first time in 1983 by Stick in this field,^[12] and today, it is used as a biomarker in genetic damage in many cases,^[13] which enables the study of nuclear changes in cells exposed to carcinogenesis in conditions before the appearance of clinical symptoms of cancer.^[14] Exploring nuclear changes in cytological specimens is performed from the mouth mucosa of patients, which is simple, noninvasive, and relatively painless.^[15] The cytomorphological changes, such as MN, are indicative of genomic damage, are biomarkers of genotoxicity, and can be tested in exfoliated cells, especially in oral mucosal cells.^[9,16] The MN represents the eccentric chromosomes or chromatin fragments radiation formed due to abnormal mitosis. The presence of an increased number of micronucleated cells indicates DNA damage.^[9,16]

As a matter of fact, the oral mucosa is the tissue that is present close to the mobile phone while in use and has chances to show possible genotoxic changes by the mobile phone radiation. Gandhi and Singh, Yadav and Sharma confirmed the genotoxicity of mobile radiation on oral mucosa.^[5,17] However, others have apparently denied the genotoxic effect.^[18-20] Considering all these disagreements, reevaluation of the effect of mobile radiation on the oral epithelium is needed.

Thus, considering the reports on the harmful effects of the waves produced by the use of cell phones as well as the limitations of studies in the field of these effects on oral mucosa, this study aimed to evaluating the MN frequency in exfoliated oral mucosal cells and its comparison between high and low mobile users.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Ethical Committee. A total of 50 male and 50 female participants between the age group of 20 and 30 years were selected from the Outpatient Department of MIDSR Dental College and Hospital, Latur, Maharashtra.

The participants were divided into the following Group I as:

- Group A – Low mobile phone users used since or <5 years and <4–5 h a week
- Group B – High mobile phone users used more than 5 years and more than 10 h a week.

Receiving or making calls was considered, while net surfing and text messaging were not included. In Group II, 65 subjects were code division multiple access (CDMA) users and 35 subjects were global system for mobile (GSM) users. These high mobile users were further divided into wired headphone users (70) and nonhead phone users (30). For all the subjects, exfoliated buccal mucosal cells were collected from the same side, in which the subject used their mobile phones the most. Only in the case of Group II, a comparative evaluation of MN was done between both sides of the buccal mucosa (right and left).

Inclusion criteria

The inclusion criteria of this study were as follows:

- Healthy individuals in the age limit of 20–28 years
- Receiving and making calls were considered
- Minimum range of 4 h or above was considered.

Exclusion criteria

The exclusion criteria of this study were as follows:

- Participants having any deleterious habits
- Participants having any oral lesions.

Before sample collection, informed written consent from each subject was collected. A detailed questionnaire was prepared to evaluate the lifestyle, dietary habits, previous history of medication, locality of residence, type of mobile used (CDMA or GSM), duration of mobile phone usage (number of years and number of hours a week), and use of headsets, wired, or not. Associated symptoms such as headaches, tingling of the skin, rashes over the skin, and the warmth of the ear were also noted.

Cell sampling and preparation

Exfoliated oral mucosa cells were collected from all participants. Before cell collection, the mouth was rinsed with 1% glacial acetic acid to remove saliva, food particles, and any other debris. A moistened wooden spatula was used to collect the exfoliated cells from the buccal mucosa and was fixed using cytofixative, and the slides were stained with Papanicolaou nuclear staining solution. Two cytological smears were collected from each participant (right and left cheek, respectively).

Scoring method

All the slides were observed under a light microscope using low magnification ($\times 10$) for screening and high magnification for counting the MN. The most commonly used method was zigzag method for screening of the slide. Cells with intact nuclei and cell boundaries were

counted. A total of 1000 cells were counted in the smear from the right and left mucosa of each participant for the presence and number of MN in each cell.

The following criteria for designating an extranuclear body as micronucleus were given by Tolbert *et al.*^[21]

- Rounded smooth perimeter suggestive of a membrane
- Less than a third of the diameter of the associated nucleus but large enough to discern shape and color
- Staining intensity similar to that of the nucleus
- Texture similar to that of the nucleus
- Same focal plane as the nucleus
- Absence of overlap with, or bridge to, the nucleus.

Statistical analysis

The percentage frequency of MN was recorded. The comparative evaluations were done using unpaired *t*-test. The values were represented in mean $\pm$ standard deviation. $P < 0.05$ was considered statistically significant.

RESULTS

There was a significant increase in the mean MN count in Group II (1.52 ± 1.176) in comparison to Group I (0.77 ± 0.815) [Table 1 and Graph 1]. There was no significant difference in the mean MN count of subjects using CDMA (0.64 ± 0.722) or GSM (0.90 ± 0.886) mobile phones [Table 2 and Graph 2]. In Group II, the MN count on the side of mobile phone use was found to be statistically significantly elevated (1.52 ± 1.176) in comparison to the opposite side (0.90 ± 0.3992) [Table 3 and Graph 3]. However, the MN mean count was found to be significantly increased in nonhead phone users (2.08 ± 1.291) in comparison to headphone users (0.96 ± 0.699) [Table 4 and Graph 4]. In Group II, mobile users without head phones who had complained about warmth around the ear, showed the highest mean count for micronucleated cells (2.847 ± 0.341).

DISCUSSION

Mobile phones, sometimes called cellular phone, are now an integral part of modern life. The mobile phone handsets are low-powered RF transmitters. Mobile phones are being used extensively throughout the world. For the year 2018, the number of mobile phone users is 4.93 billion and will reach 5 billion mark by 2019.^[22] The most conventional mobile communication system in India is GSM and CDMA. GSM Communications use frequencies of around 900 MHz bandwidth, and CDMA works on higher bandwidth, i.e., 1800 MHz.^[5] Hence, CDMA handsets may have more genotoxicity. The International Commission on Nonionizing Radiation Protection (ICNIRP) has determined the permissible

range on the level of (Specific Absorption Rate [SAR], i.e., the amount of energy absorbed per unit time per unit mass of tissue).^[23] The ICNIRP permissible range of SAR is 2.0W/kg.^[24] Nowadays, the most popular 3G network system works under the frequency range of 2100 MHz.^[25] Mobile phones radiate an average of power of 0.2–0.6 Watt/kg, 40% of which is absorbed in the head-and-neck region.^[26]

Although the radio frequency wave from emitting mobile phones is considered to be low-grade emissions in the range of 1.6–2W/kg, i.e., within the permissible range, it can still be harmful in case of long-term use for a prolonged period. Microwave radiation in the range of 2.45 GHz indicates significant DNA damage in mice model.^[27] Another study conducted by Kesari *et al.* reported an increase in MN count, caspase 3 levels, and apoptosis rate in mice model due to 3G cell phone exposure for 2 h a day for 60 days.^[28] The reported adverse effects include physiological, behavioral, and cognitive changes, as well as tumor formation and genetic damage.^[28] Long-term use of mobile radiation, even in low range, can affect the reproductive system also. Shahin *et al.* have shown decreased sperm count

Table 1: Comparison of mean micronucleus count between low and high mobile phone users

	<i>n</i>	Mean $\pm$ SD	<i>t</i>	df	<i>P</i>
High mobile users	50	2.70 $\pm$ 1.111	17.182	49	0.000
Low mobile users	50	0.98 $\pm$ 0.589	11.772	49	0.000

SD=Standard deviation

Table 2: Comparison of mean micronucleus count between code division multiple access and global system for mobiles mobile phone users

	<i>n</i>	Mean $\pm$ SD	<i>t</i>	df	<i>P</i>
CDMA users	50	1.72 $\pm$ 1.512	8.042	49	0.000
GSM users	50	1.20 $\pm$ 1.178	7.203	49	0.000

SD=Standard deviation, CDMA=Code division multiple access, GSM=Global system for mobile

Table 3: Comparison of mean micronucleus count between headphone and nonheadphone users

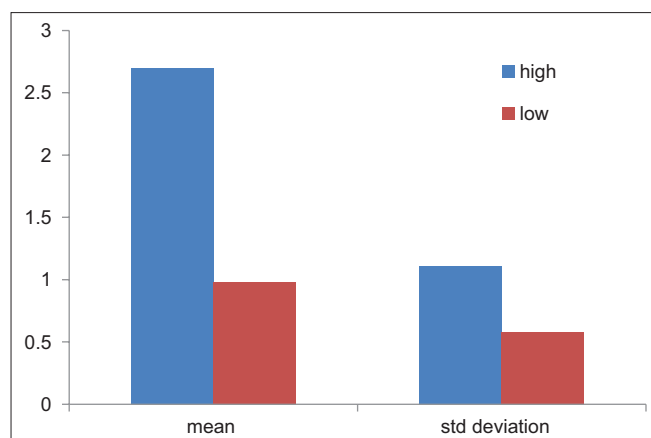
	<i>n</i>	Mean $\pm$ SD	<i>t</i>	df	<i>P</i>
Nonheadphone users	50	1.94 $\pm$ 1.202	11.411	49	0.000
Headphone users	50	1.40 $\pm$ 0.670	14.774	49	0.000

SD=Standard deviation

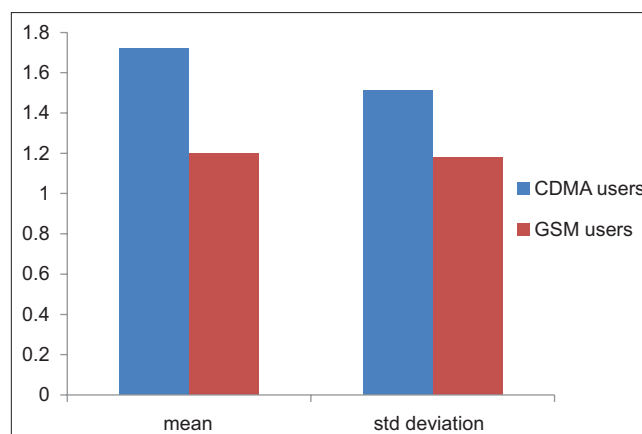
Table 4: Comparison of mean micronucleus count between same side and opposite side of mobile phone use

	<i>n</i>	Mean $\pm$ SD	<i>t</i>	df	<i>P</i>
Frequently used side	50	2.10 $\pm$ 1.313	11.308	49	0.000
Other side	50	1.10 $\pm$ 0.839	9.270	49	0.000

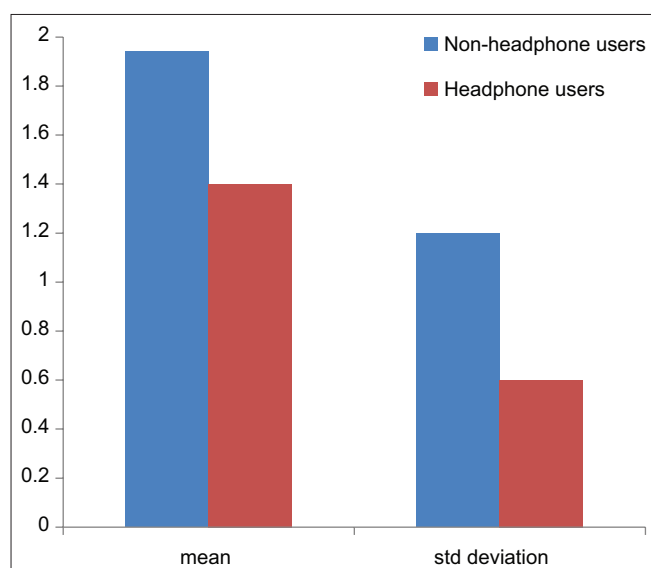
SD=Standard deviation



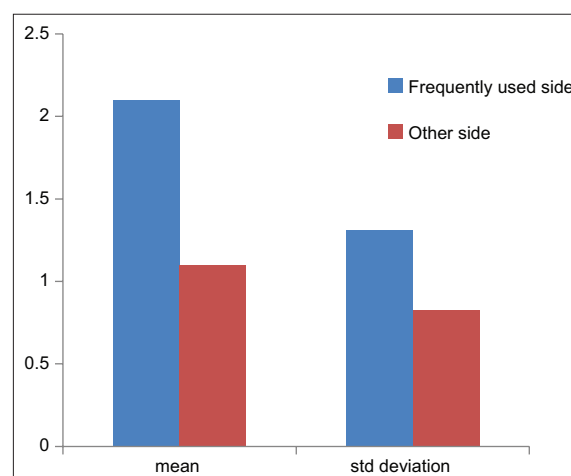
Graph 1: comparison of mean micronucleus count between low and high mobile phone users



Graph 2: comparison of mean micronucleus count between CDMA and GSM mobile phone users. CDMA = Code division multiple access, GSM = Global system for mobiles



Graph 3: comparison of mean micronucleus count between headphone and nonheadphone users



Graph 4: comparison of mean micronucleus count between same side and opposite side of mobile phone use

in male Swiss strain mice for a long time with low radiofrequency exposure by mobile phones.^[29] As the head-and-neck region is the most closely approximated area for mobile phone use, the maximum radiation effect can be expected here. In this study, micronucleus count in exfoliated buccal mucosal cells was used to evaluate the genotoxic effect of mobile phone radiation. MN count in the exfoliated cells can be used as a marker for an abnormal cell cycle as it is formed as a result of aberrant mitosis when the whole chromosome or chromatid fragment fails to reach the spindle pole. It is one of the best indicators of mitotic interference and chromosomal mutations or breakage.^[30] The MN index is preferable for mass screening as it is rapid, simple, sensitive, and cost effective. Counting of MN index is very technique sensitive, and different staining methods cause significant variations for the evaluation of its

frequency. For proper evaluation of MN, DNA-specific stain should be used, and thereby Papanicolaou stain was used in this study.^[16,31]

As stated in the result, MN count was found to be significantly higher in high mobile phone users in comparison to low users, and it directly indicates the genotoxic effect of prolonged mobile phone use for a longer period. In our study, all probable causes for the increase in the MN count were excluded (tobacco, alcohol, recent medication, systemic factors, etc.). Therefore, mobile phone radiation was expected to be the immediate and possible cause for increased MN count in higher mobile phone users. Moreover, this finding was similar to various research groups such as Yadav and Sharma^[5] found an increased frequency of micronucleated exfoliated cells in 85 cell phone users compared to 24 nonusers (controls). Gandhi and Singh^[17] also found a positive correlation between the number of MN and increasing exposure to cell phone radiation,

comparing 25 users with 25 controls. De Oliveira *et al.*,^[32] Souza Lda *et al.*,^[33] and Yadav and Sharma,^[5] same to the present study, reported a similar significant increase in nuclear anomaly in cells exfoliated from mouth mucosa. They also found that other uses of cell phones, such as messaging and listening to songs, were less toxic than talking to cell phones. However, we tried to exclude factors other than talking directly to cell phones.

Furthermore, Daroit *et al.*^[34] obtained their samples from the sites with the highest potential rates of cancer, such as the lower lip, tongue side boards, and floor of the mouth. Furthermore, Banerjee *et al.*^[4] have shown that the duration of cell phone use is very important; although cell phone frequency is within the permissible range, a long time usage has significant genetic toxicity effects. Some studies have shown that the combination of risk factors such as the effect of occupational exposure in combination with demographic social factors such as age, habits such as smoking and alcohol consumption, nutritional status, and chronic and infectious diseases can have important effects on the formation and growth of micronucleus.^[5,35]

Previously, studies have been reported on mobile phone radiations on peripheral blood lymphocytes by Vijayalaxmi *et al.*,^[36] and Agarwal *et al.*^[37] showed harmful effects of cell phone radiation on sperm function and reactive oxygen species. Nonthermal DNA breakage in human fibroblasts as well as rat granulosa cells *in vitro* due to exposure to cell phone radiations were also reported by Diem *et al.*^[38] in 2005. In this regard, Waldmann *et al.*^[39] concluded the evaluation of micronucleus in peripheral blood lymphocyte cells of people using cell phones and reported a significant increase. Despite the similarity of results, the noninvasive micronucleus assay in mouth mucosa cells compared to their evaluation in peripheral blood expresses the superiority of the present study.

On the contrary, other research groups Ros-Llor *et al.*,^[9] and Hintzsche and Stopper,^[18] have directly denied any significant increase of MN count in mobile phone users. However, Hintzsche and Stopper,^[18] exposure to other sources of electromagnetic radiation with similar frequencies, including the use of hands free, was not examined, whereas in our patient, the questionnaire asked whether they used hands free or directly touch the cell phone on their face and people who use hands free were excluded. Although the same inclusion and exclusion criteria were existing, there was a significant difference in the findings of the two studies in terms of the percentage of cells with micronucleus. This

issue can be reminiscent of the local environmental exposures.

In this study, the higher mobile phone users were also evaluated by the type of mobile phone used, i.e. CDMA or GSM. Although the CDMA phones work under higher electromagnetic frequency (1800 MHz) in comparison to GSM mobiles (900MHz), no significant MN count variability in these two groups was observed. This result indicates that, when used within the permissible range, the strength of radiofrequency radiation is not a major factor for genotoxic damage.

When the high mobile phone users were questioned for the usage of wired headphones, a significantly dramatic decrease in MN count was observed in headphone users. When headphones are used with mobile phones, it helps to keep the mobile phone away from the body, and there is no direct contact of the radiofrequency receiver with the body. The headphones also contribute to reducing the local temperature rise around the ear, and this is a commonly encountered problem by long-term mobile phone users not using headphones. A significant finding of this study was that people complaining of warmth around the ear were found to have the highest MN count, indicating that heat provides a synergistic effect on genotoxic damage, probably by the activation of heat shock proteins along with the radiofrequency radiation. It has been a proven fact that the level of heat shock protein 70 is increased as a result of radio-adaptive response.^[40] As the local rise of temperature is a direct effect of heating up of the battery of the phone or as a result of the long press of the mobile phone against the cheek, cellular response due to direct heat also cannot be ignored. Localized heat stress is proven to increase vascular permeability, making the tissue more susceptible to genotoxic stress.^[41] The local rise of temperature may even facilitate heat stress-induced mitochondrial membrane damage, release of cytochrome c, and activation of caspase-9 and-3,^[42] which in turn can be considered a prerequisite for cytotoxicity. On the other hand, the increased MN production can also be directly associated with localized hyperthermia.^[43] Hence, the increased MN count in subjects complaining of warmth around the ear may be synergistic effects of both mobile radiations induced genotoxicity and local thermal effects.

The present study has shown that amount of daily cell phone usage had a strong and significant correlation with the frequency of micronucleus-containing cells and the frequency of micronucleus in each buccal mucosa cell. Furthermore, the related factors as age and sex were not significantly correlated with these evaluated issues. Based on these results, it can be concluded that human buccal cells are likely to show increased micronucleus

cells as a result of the genotoxic effects of cell phone waves that have been chronically exposed. Although any visible oral mucosal changes were not observed, it should be taken into consideration that mobile phones are being used extensively by the general population and increased micronucleus count indicates that, in future days, it may cause visible oral lesions. The complete avoidance of mobile technology is not possible, but few precautionary steps such as keeping the mobile phone away from the body when connecting; use of headphones; and keeping the phone in switched-off mode when possible, may help us to reduce the deleterious effects of mobile radiation.

CONCLUSIONS

Mobile phones, when used for prolonged periods, can cause genotoxicity. Although, according to the SAR values, most of the mobile phones emit radiofrequency radiation within safety limits, long-term use of mobile phones shows definite signs of DNA damage. The genotoxicity accentuates when associated symptom like warmth around the ear is related. Headphone use reduces the harmful effects of mobile phone radiation. However, further studies with other nuclear modifications other than micronucleus as well as years of cell phone use, are suggested to further confirmation of the results of this study.

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Conflicts of interest

There are no conflicts of interest.

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