Micronucleus Assay in Cell Phone Users: Importance of Oral Mucosa Screening

Abstract

Background: One of the concerns of cell phone users is prolonged exposure to harmful and potentially carcinogenic waves. This study was aimed to investigate the correlation between amount of cell phone use and related factors with percentage of micronucleus containing cells. Methods: This descriptive study was conducted on selected patients referring to Islamic Azad University Faculty of Dentistry using cell phones regarding related inclusion and exclusion criteria. Papanicolaou staining method was approached for mucosal smears of samples and frequency of micronucleus containing cells and also, frequency of micronucleus in each cell were recorded for each sample; then, correlation of these findings with amount of daily cell phone usage was statistically analyzed using the calculation of Pearson correlation coefficient and preparation of regression analysis (backward) with significant level of lower than 0.05. Results: Of 100 samples, the frequency of micronucleus containing cells was 2.94% ± 1.89% and the frequency of micronucleus in each cell was 1.02% ± 1.68%. The amount of cell phone usage was significantly correlated with the frequency of micronucleus containing cells ($r = 0.70, P = 0.0001$) and also with the frequency of micronucleus in each cell ($r = 0.57, P = 0.0001$). Also, age and sex were not significantly correlated with the frequency of micronucleus containing cells ($P = 0.47$ and $0.32$) and also with the frequency of micronucleus in each cell, respectively ($P = 0.16$ and $0.27$). Conclusions: The present study showed that the increased amount of cell phone usage had a strong and significant correlation with the higher frequency of the micronucleus containing cells and the higher frequency of micronucleus in each cell in the buccal mucosa. Also, the related factors as age and sex were not significantly correlated with the frequency of micronucleus containing buccal mucosa cells.

Keywords: Cell phone, micronucleus, mouth mucosa, prevention

Introduction

Currently, more than three billion people in the world use cell phones. One of the concerns of cell phone users is the prolonged exposure to harmful and potentially carcinogenic waves through talking. With the widespread use of cell phones, even a small increase in carcinogenic risk can have a significant impact on public health. In addition, they may be exposed to other waves, such as radio waves, noise, microwave radiation, etc. Cell phones produce radiation in the frequency range of 1–2 MHz. Usually, a cancer diagnosis is made in advanced stages because there are no early obvious clinical signs. 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 and today it is used as a biomarker in genetic damage in many cases, which enables the study of nuclear changes in cells exposed to carcinogenesis in conditions prior to the appearance of clinical symptoms of cancer. Exploring nuclear changes in cytological specimens is performed from mouth mucosa of patients, which is simple, noninvasive, and relatively painless. According to studies, micronucleus assay is one of the most sensitive markers of DNA damage. This marker has been used for genotoxicity studies of various chemicals. It is also used to assess the effects of genetic damage caused by occupational and environmental factors in human epidemiological evaluation and animal testing. Many research has been done on the disadvantages and harmfulness of cell phone waves that yield different results. In a study by Oliveira et al., it is stated that micronucleus is a good parameter to identify high-risk individuals

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How to cite this article: Ghandehari M, Sadri D, Farhadi S. Micronucleus assay in cell phone users: Importance of oral mucosa screening. Int J Prev Med 2021;12:125.

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Access this article online
Website: www.ijpvmjournal.net/www.ijpm.ir
DOI: 10.4103/ijpvm.IJPVM_489_19
Quick Response Code:
and to determine the biological conditions of oral lesions.\textsuperscript{[14]}

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 was aimed to investigate the correlation between cell phone utilization and related factors and micronucleus frequency of mouth mucosa cells in patients referring to Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University at 2018.

Methods

This study was a descriptive and cross-sectional study with purpose-based sampling. After approval of ethical consideration from University ethical approval committee, the statistical population was selected from patients referring to Dentistry Faculty of Islamic Azad University using cell phones. Inclusion criteria were living in Tehran who used cell phones to speak directly (without hands free) without history of radiotherapy, alcohol and tobacco and medicines use, systemic disease, recent viral infection, occupations in contact with chemicals, and presence of pathological lesions in the mouth mucosa to eliminate the effect of confounding factors. 100 samples were considered in this study and after obtaining consent from patients about their agreement to cooperate in the research, sampling was made from the buccal mucosa of dominant side of cell phone use with damp tongue depressors (abaisse-longue) and spread on small clean glass slides. The smears prepared on the microscopic slide were fixed with a patofix spray (Iran Teb Co.) After fixation and drying at room temperature, Papanicolaou staining was used to evaluate cells containing micronucleus.\textsuperscript{[15,16]} Cellular examination was made by Japan-made light microscope (Nikon Ys 100) with 400 times magnification. 500 cells were examined for each sample and the frequency of micronucleus containing cells was reported as percentage. The number of micronucleus in each cell was also counted and the final result was reported too [Figure 1].\textsuperscript{[16]}

Statistical evaluation

Pearson correlation analysis was obtained to investigate the correlation of daily amount of cell phone utilization with the frequency of micronucleus in buccal mucosa cells and also with the frequency of micronucleus in each cell. Although the role of other factors as age and sex was determined by regression analysis (backward method).

Results

100 stained specimens were statistically analyzed and the following findings were obtained.

Table 1 demonstrates the given data of studied samples as the frequency of micronucleus containing cells was 2.94% ± 1.89% and the frequency of micronucleus in each cell was 1.02% ± 1.68%. But, as shown in Table 2, based on the results of statistical analysis, the amount of daily cell phone usage was significantly correlated with the frequency of micronucleus containing cells ($r = 0.70, P = 0.0001$).

Also, the amount of daily cell phone usage was significantly correlated with the frequency of micronucleus in each cell ($r = 0.57, P = 0.0001$).

In addition, Diagram 1 shows the correlation between cell phone usage and frequency of micronucleus containing cells. It is clear that most of the points were on the same line and few were scattered, indicating a strong correlation between the two factors of cell phone usage and micronucleus frequency.

In the next step, the correlation of age and sex with frequency of micronucleus containing cells and also with frequency of micronucleus in each cell was investigated and the following results were obtained:

Statistical analysis was determined that age was not significantly correlated with the frequency of micronucleus containing cells ($P = 0.47$) and also with the frequency of micronucleus in each cell ($P = 0.16$). In addition, sex was not significantly correlated with the frequency of micronucleus containing cells ($P = 0.32$) and as well with the frequency of micronucleus in each cell ($P = 0.27$).
Table 1: The evaluated frequencies of the studied samples

| The evaluated frequencies                          | Minimum frequency | Maximum frequency | Mean±S.D |
|---------------------------------------------------|-------------------|------------------|----------|
| Cell phone usage (hour)                           | 0.11 h            | 7 h              | 1.72 h±1.5 |
| Frequency of micronucleus containing cells (percentage) | 0.24%             | 9.51%            | 2.94%±1.89% |
| Frequency of micronucleus in each cell (number)   | 0.30              | 5.90             | 1.68±1.02 |

Table 2: Statistical evaluation of the correlation of cell phone usage with frequency of micronucleus containing cells and frequency of micronucleus in each buccal cell

| Statistical evaluation | Correlation of cell phone usage with frequency of micronucleus containing cells | Correlation of cell phone usage and frequency of micronucleus in each cell | Correlation coefficient (r) | P       |
|------------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------|---------|
|                        |                                                                                  |                                                                              | 0.70                        | 0.0001  |
|                        |                                                                                  |                                                                              | 0.57                        |         |
|                        |                                                                                  |                                                                              |                             |         |

Discussion

According to studies, micronucleus is introduced as one of the most sensitive markers of DNA damage and used for genotoxicity studies of various chemicals. It is also used to assess the effects of genetic damage caused by occupational and environmental factors in human epidemiological evaluation and also animal experimental studies. Many studies as Oliveira et al. study have been done on the disadvantages and effects of cell phone waves that yield different results. Oliveira et al., Souza et al., and Singh et al. 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.

In this regard, Waldman 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.

Lior et al. and Hintzsch and Stopper, in contrast to the present results, did not observe any significant difference in the amount of cell phone use with micronucleus. Although the study was a cohort study, despite the current study, sampling was approached randomly even left or right side not corresponding to predominant using side.

Also, in the study of Hintzsch and Stopper, which did not report significant results in this field, exposure to other sources of electromagnetic radiation with similar frequencies, including the use of hands-free, was not examined, whereas in our patient questionnaire asked whether they use 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 percentage of cells with micronucleus. This issue can be reminiscent of the local environment exposures.

In addition, unlike the present study, Pellicoli et al. evaluated other nuclear anomalies other than micronucleus including binucleated cell, broken egg, karyolysis, picknosis, and karyorrhexis and reported the association of these changes with smoking and alcohol and oral lesions, which are the strengths of this study. Also, the study showed that evaluation of cytogenic issue as micronucleus and broken egg’s abnormalities is useful for monitoring the progression of oral cancer.

Also, Batista et al. 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, Banerje et al. 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.

In this regard, it is important to know that cell phones and radio base stations that transmit and receive electromagnetic signals are known as electromagnetic radio fields (EMF-RF). EMF-RF do not have enough energy to directly break the DNA’s chemical bonds, but may indirectly disrupt the DNA repair mechanism by causing reactive oxygen species, leading genotoxic damage to cells.

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. Also, 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 which have been chronically exposed. In addition, increasing short-term exposure time, such as increasing cell phone usage per day, can exacerbate the increase in mouth...
mucosa cell nuclear changes. 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.

**Declaration of patient consent**

The authors certify to obtain the appropriate patient consent forms for participation in this research.

**Acknowledgment**

This article is based on General Dentistry Thesis No.:25911, at 2019 Faculty of Dentistry, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran and we are appreciated who were cooperated in this study, especially Dr. Mohammad Javad Kharazifard for Statistical evaluation of findings.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**Received:** 11 Jan 20  **Accepted:** 17 Apr 20  **Published:** 29 Sep 21

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