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Mutagenicity in oral cells of individuals exposed to radiofrequency generated by different smartphones

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Aim: This study aimed to investigate whether non-ionizing radiation emitted by smartphones is likely to cause genotoxic effects on oral epithelial cells. **Methods:** Thirty adults were distributed into two groups according to the mobile phone brand used, namely Samsung (Samsung, Seoul, South Korea) and Apple (Apple, California, USA). The material was collected with gentle swabbing of the right and left buccal mucosa using a cervical brush, then the micronucleus test was performed. **Results:** The Mann-Whitney test with a 5% significance level did not reveal statistically significant differences in micronuclei frequency between the exposed and non-exposed sides (p=0.251). The different brands do not seem to cause risks of inducing genetic damage because there were no statistically significant differences between them (p=0.47). **Conclusion:** Therefore, our results suggest no correlations of micronuclei frequency in the exposed buccal cells of mobile phone users at the exposure standard levels observed.

Keywords: Radio waves. Micronucleus tests. Mutagenicity tests.

Introduction

The advent of globalization and the importance of communication networks in this context has increased the use of technologies such as smartphones exponentially all over the world. More than two-thirds of the world population, meaning over five billion people, are mobile telephony subscribers. This technology is based on the exchange of signals between smartphones and base stations through electromagnetic waves known as radiofrequency electromagnetic fields (RF-EMF)[1,2]. International organizations are responsible for establishing guidelines on limits of exposure to radiofrequency. According to the International Commission on Non-Ionizing Radiation Protection[3] (2009), the acceptable limit, which is used in several countries, is a maximum of 300 GHz. The rate at which RF-EMF is absorbed by the human body is called specific absorption rate (SAR), which is a standardized unit that measures the impact of radiofrequency electromagnetic waves on the human body and is expressed as Watt/kg. The maximum legal SAR level limited to any mobile phone is 1.6 Watt/kg[2]. Therefore, it is important to investigate the effects of exposure to these radiofrequency values.

Radiofrequency exposure limits are necessary because the human body absorbs a part of electromagnetic waves, implying serious biological risks. Biological consequences such as a higher risk of neurological and auditory diseases have been reported in the literature but the results are contradictory[4,5]. The effects of these radiations are classified into thermal and non-thermal[6]. The first case happens because non-ionizing radiation can release a sufficient amount of energy to warm up the biological tissue, and serious damage may occur when exceeding the limit levels[6,7]. According to Christ and Kuster[8] (2005), several factors influence the amount of radiation absorbed by the head of users, namely the power required to transmit and receive the signal from the radio base station (tower), the model of the antenna, phone design, and the positioning relative to the head.

The energy emitted by RF-EMF is not sufficiently capable of causing direct DNA damage but may interfere with the genome through indirect mechanisms such as the production of reactive oxygen species, chromatin disorganization, and impairment of DNA repair[9]. Given the carcinogenic potential of RF-EMF in human cells, classified as a Group 2B agent[10], the study of potential mutagenic alterations in the oral epithelium of individuals exposed to this radiation is relevant.

De Oliveira et al.[4] (2017) reported that DNA damage can trigger important cellular changes such as senescence, death, or malfunction. These genotoxic changes can be diagnosed with methods such as the micronucleus (MN) test, which allows observing chromatin fragments from chromosomal breakage due to clastogenic or aneugenic events also classified as genotoxicity biomarkers[11].

The MN test, performed through exfoliative cytology of the oral epithelium, is a useful and minimally invasive diagnostic tool for assessing genetic damage in humans[12]. According to Ros-Llor et al.[13] (2012), the test is fast and practical, considering that oral mucosa cells are easier to collect than others, such as blood cells.

Considering the growing number of smartphone users, the risk of genetic damages may contribute to implementing measures to help eradicate long-standing misconceptions about the radiation emitted by these devices. Thus, the present study aimed to evaluate, by microscopic observation using the MN test, whether non-ionizing radiation emitted by mobile phones of different brands caused mutagenic effects on oral mucosa cells.

Materials and Methods

Study population

Thirty adults (12 men and 18 women) were selected for the study. The sample size calculation was based on the studies by Daroit et al.[5] (2015), Souza et al.[9] (2014), and Yadav and Sharma[14] (2008), in which the mean and standard deviation values of 3.75 and 3.791, respectively, were used for the variable of "total micronucleus". Considering a study with 90% power and $\alpha = 0.05$, the acceptable sample size was at least 13 participants in each group.

Volunteers who reported not using a mobile phone or having no preference of side (right and left) when using the device were excluded. Individuals who reported using non-traditional mechanisms to answer calls such as headsets, hands-free devices, and Bluetooth were not included in the survey. Other exclusion criteria were the presence of diseases such as diabetes and anemia; use of medications; reports of facial trauma; pregnancy; chronic use of alcohol or drugs; smoking; use of orthodontic appliances; exposure to oral X-rays one month before the study; use of mouthwashes, and use of tooth-desensitizing or bleaching agents 21 days before the study.

Volunteers with the following characteristics were included in the study: male or female individuals aged between 20 and 30 years and individuals with good general and oral health without changes in the oral mucosa.

All participants signed the informed consent, and the study was approved by the Human Research Ethics Committee (CAAE: 53233716.5.0000.5546), following the Declaration of Helsinki. The participants included responded to a questionnaire on sociodemographic data, past medical history, family history, habits (e.g., alcohol and tobacco consumption), diet, and history of exposure to RF-EMF (time using smartphones, the number of minutes a day using smartphones, and side of the face preferred when using the device). The groups were divided according to the questionnaire answers and two mobile phone brands were compared: Samsung (head SAR value = 0.52 W/kg; body SAR value= 0.99W/kg) (Samsung, Seoul, South Korea) and Apple (head SAR value = 1.2W/kg; body SAR value= 1.13W/kg) (Apple, California, USA).

Collection of material

After a mouth rinse with water, cells were collected by gentle swabbing of the right and left buccal mucosa with a Cytobrush cervical brush (Adlin, Jaraguá do Sul/SC-Brazil). The cells were transferred to a vial containing a fixative solution

(Sra Medical, Balneário Camboriú/SC-Brazil). Then, they were homogenized in a vortex shaker at speed four for 30 seconds (NI 1059 - Novainstruments Equipamentos para Laboratórios Ltda., Piracicaba/SP-Brazil), centrifuged for 10 minutes at 1000 rpm, 130 × *g* (Baby I 206 – FANEM, Guarulhos/SP-Brazil), and finally placed on glass slides and allowed to dry at room temperature for about one hour. The cells were fixed on the glass slides with 80% ethanol for 48 hours before staining.

After drying, the slides were stained with hematoxylin-eosin (HE), an acid-basic stain that produces a contrast between the cytoplasm and the nucleus. First, the samples were exposed to hematoxylin, a basic dye that binds to substances containing acid groups. Then, the samples were exposed to eosin, a weak acid colorant that stains basic structures. Considering this characteristic, HE has a high affinity with nuclear cells presenting great blue and pink colorations[15].

Analysis of slides

An oral pathologist with over 10 years of experience performed a blind evaluation. Calibration was performed with the joint analysis of five slides, totaling approximately 6,000 cells. The intraclass correlation coefficient (ICC) value was 0.79, indicating excellent agreement.

An Olympus CX31 transmitted light microscope model (São Paulo/SP-Brazil) was used for slide analyses. The slides were analyzed from left to right and top to bottom with a 40× objective. Then, an immersion objective was used for micronucleus analysis. Micronuclei were searched in 2,000-cell nuclei per cytological smear[15,16], and an additional 2,000 cells were analyzed when the frequency of micronuclei was higher than 2%. The micronuclei were identified according to the criteria by Sarto et al.[17] (1987) for measuring DNA damage/genotoxicity.

Data analysis

The results of the microscopic analysis of the cell counts of the oral mucosa exposed to radiofrequency radiation were tabulated in Microsoft Excel, version 2010 for Windows 64-bit (Microsoft Corporation, Redmond, WA, USA). The Shapiro-Wilk test was used to verify the normality of distribution. As a non-Gaussian distribution was found, the Mann-Whitney test was used. A t-test was used to compare the mobile phone brands, as Gaussian distribution was observed in this case. The statistical tests were performed using the R software with the Rcmdr package, version 3.2.1 for Windows 64-bit (The R Foundation, Vienna, Austria). A 5% significance level was set for all statistical analyses.

Results

Cells of the right and left buccal mucosa of 30 individuals (15 users of Apple and 15 users of Samsung mobile phones) were evaluated, resulting in 60 samples. Table 1 shows the most important characteristics of the study population. There were male (40%) and female (60%) participants aged between 20 and 30 years. The total period of exposure in this study was predominantly in the range of over 10 years (73%).

Table 1. Characteristics of the study population.

Patients: n	30
Age: mean	23.93
Sex: n (%)	
Male	12 (40)
Female	18 (60)
Time of exposure to mobile phones: n (%)	
< 5 years	0 (0)
5 – 10 years	8 (27)
> 10 years	22 (73)
Mobile phone use (h/week): n (%)	
0	0
0 - 2	26 (87)
2 - 4	4 (13)
Hand used to answer calls: n (%)	
Right	28 (93)
Left	2 (7)

According to the Mann-Whitney test, the micronuclei count was not statistically different between exposed and non-exposed sides (p = 0.251) (Figure 1). Differences between brands were not statistically significant (Table 2).

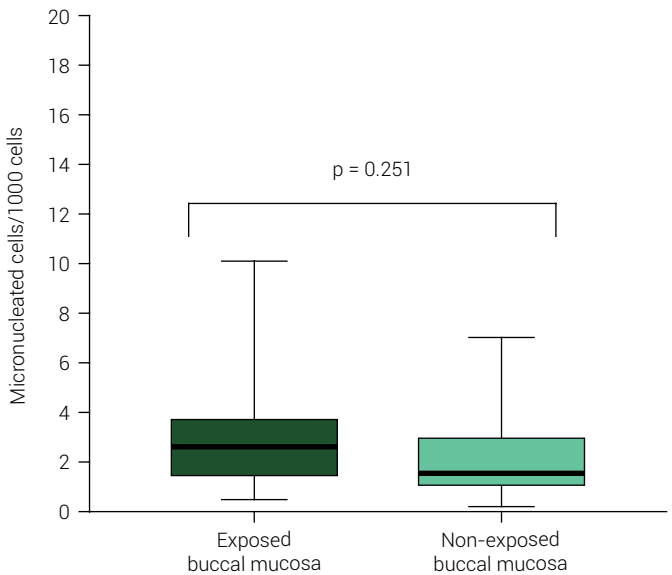

Figure 1. Median micronuclei count (maximum and minimum) regardless of brand (Mann-Whitney test).

Table 2. Micronuclei count according to mobile phone brand (t-test).

	Micronuclei		
	Mean	SD	p-value
Apple	2.70	±1.45	0.47
Samsung	3.23	±2.46	

SD: standard deviation.

Discussion

In the global communication era, mobile phones are often used and some assumptions regarding their side effects are questioned. The present study aimed to evaluate, with the MN assay, whether the radiation emitted by mobile phones can cause mutagenic effects on oral epithelium cells. The results presented in this study suggest that ionizing radiation associated with mobile phones does not induce the formation of micronuclei in buccal cells at the exposure levels observed. These results agree with some studies[3,4,9,17] that demonstrated that using smartphones does not cause genotoxicity, considering that exposed and non-exposed sides did not show statistically significant differences.

However, the literature on this topic is controversial, as other studies with exfoliated cells[5,14,18] showed a significantly higher number of micronuclei, indicating that mobile phones may cause genotoxicity in contrast with the results presented in this study. Daroit et al[5]. (2014) showed a slight increase in the number of micronucleated cells in the oral mucosa of individuals who used their phones more than 60 minutes per week over eight years. Banerjee[18] et al. (2016) investigated micronuclei count in mobile phone users, comparing 150 "low-frequency users" (less than 3 h/week using cell phones) with 150 "high-frequency users" (more than 10 h/week). Considering the "high-frequency users" group, a comparative evaluation of both sides of the buccal mucosa was performed, which showed a statistically significant higher frequency of micronuclei in exfoliated buccal cells of the exposed side than those of the contralateral side.

Yadav and Sharma[14] (2008) found twice as many micronuclei in mobile phone users than in non-users and reported an increased frequency of micronuclei related to the total time of exposure. However, they used orcein, a non-DNA-specific stain that may stain DNA containing micronuclei and other artifacts not associated with genomic instability, which could imply false-positive micronuclei count.

The present study used hematoxylin and eosin (HE), an acid-basic stain that produces a contrast between the cytoplasm and the nucleus and may mark MN frequency considerably[19]. The samples are first exposed to hematoxylin, a basic dye that binds to any substance containing acid groups, such as the phosphate groups in DNA structure, and to nuclear proteins with a negative charge. Then, samples can be exposed to eosin, a weak acid colorant that stains basic structures. The basophil structures such as nuclei are stained in blue with hematoxylin, while eosin stains acidophil structures such as collagen fibers in pink. Some complications may occur during colorant precipitation, which could facilitate a false-positive result. However,

if there is sufficient precaution during slide preparation and staining, this is a reliable method for MN detection[15].

The MN assay is often used in the oral mucosa due to rapid renewal, and the collection of oral cells involves minimal invasion and high representation of the epithelial tissue[4,12]. The genetic analysis with exfoliated epithelial cells of the oral mucosa provides several advantages because it is the primary target of exposure and the minimally invasive technique allows monitoring populations exposed to genotoxic agents and the association of lifestyles with the epithelial damages detected[12]. Accordingly, the micronucleus assay with exfoliated cells was chosen because it is well established as a reliable assessment test.

There were no statistically significant differences between the brands compared. Each cell phone model has its specific absorption rate (SAR), which is the amount of energy absorbed per unit mass of tissue during a given time interval, determined by the ICNIRP[3] (2009). The acceptable rate used in Brazil is two watts per kilogram of body weight[5]. The SAR for each device used in the study was lower than the recommendations of the responsible institution.

Other features are directly linked to the increase in MN count. As increasing age (> 40 years) and cigarette consumption (> 40/day) exert a highly significant influence on micronucleus frequency[15,20], the participants of the present study were carefully selected to exclude biases. Reducing the age difference of patients was attempted, establishing an age range of 20 to 30 years, and smoking patients were excluded. Regarding the count of the number of cells, it must be scored in order to obtain statistically results needs to be addressed. Tolbert et al.[21] (1992), recommended scoring at least 1000 cells per plate, which represents a great method for determining the frequency of all the various types of cells. Most recent studies, have scored between 1000 and 3000 cells, which are in accordance to the methodology adopted in our study[4,9,16,19,22].

A few studies[3,4,17] have analyzed the potential correlation of micronuclei frequency with demographic data (sex, age, and place of birth), social origin, and environmental factors (occupation, duration and recent work changes, proximity of homes to helipads or airports, alcohol and tobacco consumption, diet, vitamin supplementation, family history of cancer, chronic medication, and risk factors). However, none showed statistically significant results, which agrees with most studies using the MN test for the oral mucosa.

Several investigations involving the use of mobile phones are limited due to the challenge to establish a control group[4] because the vast majority of the population uses mobile phones, making it nearly impossible to find a sufficient number of individuals who do not use cell phones regularly. Due to this difficulty, the present study used the side of the face that was not preferred when answering calls as the control group.

The side and the duration of mobile phone use are subject to errors associated with self-reporting methods because underestimations and overestimations are common. Although bias is a tangible obstacle to epidemiological research, self-reporting is often the only alternative available to evaluate certain variables. Considering the increase in the number of mobile phone users and the dilemma regarding their

biological consequences, the present study is important and further research is still required to better elucidate such effects.

In conclusion, this study suggests that the mobile phone brands investigated do not have genotoxic potential when comparing MN frequency between the exposed buccal mucosa side and the non-exposed side.

Data availability

Datasets related to this article will be available upon request to the corresponding author.

Conflict of Interests

None.

Author Contribution

	Contributor 1 Liciane	Contributor 2 Itana	Contributor 3 Marcos	Contributor 4 Andrea	Contributor 5 Silvia	Contributor 6 Wilton
Conception of the work	√	√	√			√
Design of the work	√	√	√			√
Data acquisition	√	√	√			√
Data analysis	√	√	√	√	√	√
Data Interpretation	√	√	√	√	√	√
Manuscript preparation/ Work Draft	√	√	√	√	√	√
Manuscript review/ Work Review	√	√	√	√	√	√
Final approval of the version to be published	√	√	√	√	√	√

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