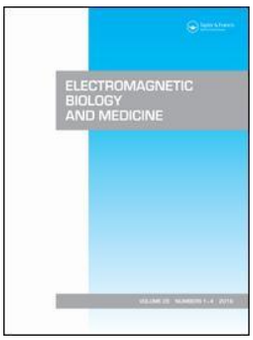

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ORIGINAL ARTICLE

Assessment of nuclear abnormalities in exfoliated cells from the oral epithelium of mobile phone users

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Abstract

Transmission and reception of mobile telephony signals take place through electromagnetic wave radiation, or electromagnetic radiofrequency fields, between the mobile terminal and the radio base station. Based on reports in the literature on adverse effects from exposure to this type of radiation, the objective of this study was to evaluate the genotoxic and cytotoxic potential of such exposure, by means of the micronucleus test on exfoliated cells from the oral epithelium. The sample included 45 individuals distributed in 3 groups according to the amount of time in hours per week (t) spent using mobile phones: group I, t≤5 h; group II, t≤1 h and 5 h; and group III, t 1 h. Cells from the oral mucosa were analyzed to assess the numbers of micronuclei, broken egg structures and degenerative nuclear abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) or necrosis (karyolysis in addition to these changes). The occurrences of micronuclei and degenerative nuclear abnormalities did not differ between the groups, but the number of broken egg (structures that may be associated with gene amplification) was significantly greater in the individuals in group I ($p \leq 0.05$).

Keywords

Apoptosis, broken eggs, micronucleus, mobile phone, necrosis

History

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Introduction

The numbers of users of mobile telephony have been increasing significantly all around the world. In 2010, according to estimates from the International Telecommunications Union (ITU), 630 million mobile telephone ''lines'' were in operation, reaching around 5.3 billion subscriptions and signal coverage encompassing 90% of the world's population (ITU, 2011).

Mobile telephones and their radio base stations transmit and receive signals using electromagnetic waves, also known as electromagnetic radiofrequency fields (EMF-RF). Depending on the technology used, mobile phones operate at frequencies ranging from 450 MHz to 2500 MHz. Around 80% of the mobile phones in use are based on the Global System for Mobile Communication (GSM), using the Time Division Multiple Access (TDMA) technology (GSM World, 2011).

The idea that there may be effects on biological systems from EMF-RF has been debated not only within the scientific community but also by the general public (Blank and Goodman, 2009; Cervellati et al., 2009; Funk et al., 2009; Khurana et al., 2009; Lin, 1997). The possibility that exposure to EMF-RF may modify the genetic material is one of the main themes of this debate (Hintzsche and Stopper, 2010; Phillips et al., 2009; Verschaeve, 2009; Yadav and Sharma, 2008).

EMF-RF does not have enough energy to cause direct breakage of the chemical bonds of DNA, but it may act indirectly through generating reactive oxygen species (Lai and Singh, 2004) and DNA repair mechanism disorders (Zhijian et al., 2010). However, the reports in the literature on genotoxic action due to radiofrequency fields produced by mobile phones have been a source of controversy (Hintzsche and Stopper, 2010; Ruediger, 2009; Verschaeve, 2009; Yadav and Sharma, 2008).

Given the large numbers of mobile phone users and the relationship between genetic damage and cancer, investigation of associations between such damage and mobile phone use may contribute toward implementing measures to prevent this disease (Hardell et al., 2007; Khurana et al., 2009; Phillips et al., 2009).

Among the various methodologies available for this type of investigation, the micronucleus test in exfoliated cells from the oral epithelium cells has been widely used (Bonassi et al., 2007; Casartelli et al., 2000; Hintzsche and Stopper, 2010; Leal-Garza et al., 2002; Yadav and Sharma, 2008) because of its reliability and other advantages highlighted by Stich et al. (1983).

Micronuclei are structures resulting from chromosome fragments or entire chromosomes that, because of failure to

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bind to the spindle, are not included in the nucleus of the daughter cells during cell division (Holland et al., 2008). They, therefore, reflect occurrences of aneugenic and clastogenic damage and, according to Bonassi et al. (2007), they are markers of cancer risk. According to Tolbert et al. (1991, 1992), assessment of degenerative abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) and necrosis (condensed chromatin, karyorrhexis, pyknosis and karyolysis) should be done concomitantly, because at excessive levels, they indicate genotoxic and cytotoxic effects, respectively. In addition, the presence of broken egg structures needs to be assessed: the real significance of these structures remains a target for discussion (Fenech, 2002; Fenech and Crott, 2002).

The micronucleus test on epithelial cells exfoliated from the oral mucosa is appropriate for evaluating the effects from EMF-RF emitted by mobile phones because this mucosa lies within the exposure area (Krewski et al., 2001; Yadav and Sharma, 2008).

The aim of this study was to evaluate the genotoxic and cytotoxic potential of exposure to the radiofrequencies emitted by mobile phone devices, by means of the micronucleus test on cells exfoliated from the oral epithelium.

Materials and methods

Sample

The sample analyzed included a total of 45 individuals, distributed into 3 groups of 15 individuals according to the amount of time in hours per week (t) that they spent using mobile phones:

Group I: more than 5 h (t 5 h);
Group II: more than 1 h and up to and including 5 h (1 t 5 h);
Group III: 1 h or less (t 1 h).

A questionnaire asking about age, mobile phone use, smoking and/or alcohol consumption habits and exposure to known genotoxic agents (chemical or physical mutagens) was applied by one of us with the aim of characterizing the sample.

Individuals were not included in the sample if they: (1) lived less than 30 meters from radio base stations; (2) used earpieces while on the phone; (3) reported oral lesions; (4) said that they had a history of cancer in the oral mucosa; (5) had undergone radiography less than 2 months earlier; (6) were smokers; (7) consumed alcoholic drinks at frequencies greater than once a week; and (8) referred exposition to chemical mutagens.

Cytological preparations

The cells were collected by gently scraping the oral mucosa (on the side on which the participant said that he or she most frequently used the mobile phone), using an endocervical brush. From the material obtained, smears on glass slides were produced, with the addition of a drop of physiological serum solution (0.9% NaCl). This material was fixed in a solution of methanol and acetic acid (3:1) and, 24 h later, it was stained using periodic acid-Schiff reagent and counterstained with 1% fast green.

Cytogenetic analysis

The analysis on the micronuclei, broken egg structures and degenerative nuclear abnormalities (Figure 1) was done under an optical microscope on a minimum of 1000 cells, in a blind test in relation to the data of the questionnaire, in accordance with the protocol suggested by Tolbert et al. (1991, 1992).

Statistical analysis

The statistical analysis on occurrences of micronuclei and nuclear abnormalities was done using the Conditional Test for Comparison of Proportions in Situations of Rare Events (Braganc,a-Pereira, 1991). The mean age of the participants in each group was analyzed using ANOVA.

Ethical issues

In compliance with Resolution 196/96 of the National Health Board, the activities of this study were started after approval for the project had been granted by the Ethics Committee for Research on Human Beings of Feira de Santana State University (Protocol No. 081/2010, CAAE 0079.0.059.000-10).

Results

Sample characteristics

The mean ages standard deviation (sd) of the participants in groups I, II and III were, respectively, 32.73 9.03, 31.87 9.78 and 32.53 9.61. ANOVA did not reveal any significant difference (p 0.05). With regard to gender, groups I and II were constituted by eight women and seven men, while group III comprised seven women and eight men. The groups were also similar regarding their alcohol consumption habits.

The mean length of time spent on conversations using mobile phones per week (approximate sum of calls made and received) in group I was 26.33 9.61 h; in group II,

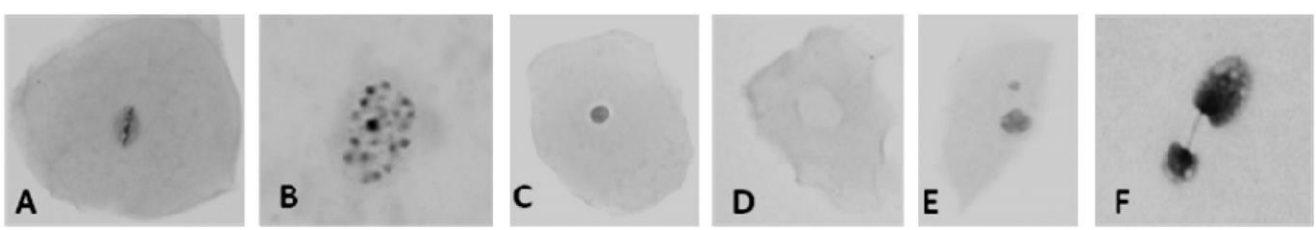

Figure 1. Photomicrograph of cells exfoliated from the oral mucosa, presenting condensed chromatin (A), karyorrhexis (B), pyknosis (C), karyolysis (D), micronucleus (E) and broken egg (F).

2.91 1.16 h; and in Group III, 0.53 0.29 h. Most of the participants (75.55%) said that they preferred to take calls using one ear rather than the other. All the individuals were using Global System for Mobile Communication (GSM) technology. Data about phone frequency used by the subjects and SAR values of mobile phones are presented in Table 1.

Analysis on micronuclei and nuclear abnormality

The statistical evaluation on occurrences of micronuclei and abnormalities indicative of apoptosis and necrosis did not reveal any significant difference between the groups (Tables 2, 3 and 4).

There were significantly greater occurrences of broken egg structures in group I than in the other two groups, which did not present any statistical difference between each other (Table 5).

Table 1. Phone frequency used by the subjects and SAR values of mobile phones.

Subject	Group	Frequency ranges (MHz)	SAR values (W/Kg)
1	I	850/1900	0.54
2	I	850/900/1800	No obtained
3	I	1900	0.344
4	I	850/1900	1.38
5	I	1900	0.344
6	I	850/1900	0.93
7	I	1800	0.72
8	I	850/1800/1900	No obtained
9	I	850/900/1800	1.23
10	I	900/1800	0.63
11	I	850/1900	1.0
12	I	1800	0.723
13	I	900/1800	0.48
14	I	850/900/1800/1900	0.78
15	I	850/900/1800	1.33
16	II	900/1800	0.90
16	II	850/900/1800	1.33
18	II	1800	0.8
19	II	1800	0.70
20	II	900/1800	0.640
21	II	900/1800	0.723
22	II	1800	1.08
23	II	1800	1.33
24	II	1800	0.7
25	II	900/1800	1.38
26	II	900/1800	0.723
27	II	1800	0.797
28	II	850/1900	0.73
29	II	1800	0.452
30	II	850/900/1800	No obtained
31	III	850/1900	0.99
32	III	1800	0.957
33	III	850/1900	0.85
34	III	850/900/1800/1900	0.868
35	III	850/900/1800	No obtained
36	III	1900	1.11
37	III	1800	0.36
38	III	900/1800	0.96
39	III	1900	No obtained
40	III	1900	1.22
41	III	1800	No obtained
42	III	1800	1.08
43	III	1800	0.875
44	III	1800	0.66
45	III	1800	0.70

Discussion

Among the possible adverse effects from exposure to electromagnetic fields, induction of cancer is certainly the most serious effect (Calvente et al., 2010; Hardell et al., 2007; Khurana et al., 2009). Today, mobile telephony is one of the main sources of exposure to EMF, and specifically EMF-RF, because of the widespread use of mobile phones and the proximity of the antenna of the device to the regions of individuals' hands, necks and, especially, heads, while they are making calls (Krewski et al., 2001).

The results obtained from this study are in agreement with the findings of McNamee et al. (2002), Scarfi et al. (2006) and Stronati et al. (2006). None of these studies showed any potential for EMF-RF emitted by mobile phones to cause chromosomal damage in the form of micronuclei.

However, in an evaluation on occurrences of micronuclei and micronucleated cells among cells exfoliated from the oral mucosa of 85 mobile phone users and 24 nonusers in the city of Kurukshetra (India), Yadav and Sharma (2008) observed that there was higher frequency of both micronuclei and micronucleated cells among the users. They also reported that there was an association between occurrences of micronuclei and duration of exposure (in years).

Table 2. Occurrences of micronuclei in the groups.

Group	Micronuclei observed	Micronuclei expected	Total number of cells	χ^2
I	3.00	3.00	15.903	0.0001
II	3.00	2.99	15.850	Df¼ 2
III	3.00	3.00	15.955	p40.90

Table 3. Data relating to occurrences of apoptosis.*

Group	Observed	Expected	Total number of cells	χ^2
I	580.00	546.34	15.903	4.3971
II	509.00	544.52	15.850	Df¼ 2
III	550.00	548.13	15.955	p40.05

*P condensed chromatin, karyorrhexis and pyknosis.

Table 4. Data relating to occurrences of necrosis.**

Group	Observed	Expected	Total number of cells	χ^2
I	581.00	548.68	15.903	4.1365
II	512.00	546.85	15.850	Df¼ 2
III	553.00	550.47	15.955	p40.05

**P condensed chromatin, karyorrhexis, pyknosis and karyolysis.

Table 5. Data relating to occurrences of broken egg structures.

Group	Obs.	Exp.	] Cells	χ^2	Partitions of χ^2 (df¼ 1)
I	11.00	5.33	15.903	9.1277	(I) x (II)¼ 4.6245; p50.05
II	3.00	5.31	15.850	Df¼ 2	(I) x (III)¼ 6.2904; p50.05
III	2.00	5.35	15.955	p50.50	(II) x (III)¼ 0.2067; p40.05

Obs., observed; Exp., expected;], total number of cells.

In 2010, Hintzsche and Stopper conducted a study with the aim of ascertaining whether the results obtained by Yadav and Sharma (2008) could be confirmed in another population (Wuerzburg, Germany). As well as including a larger number of individuals in the sample (118 users and 13 nonusers), these authors made use of a DNA-specific staining medium. The frequencies of micronuclei did not differ in relation to use or in relation to time of use. These authors concluded that the differences in results between the two studies could be due to the greater sample size and the use of the DNA-specific staining medium, although they could also be a reflection of genotype variations between these different populations.

The results from this study reinforce the idea that using a DNA-specific staining medium promotes more trustworthy results, given that the results were similar to those of Hintzsche and Stopper (2010), despite being obtained from a smaller sample among a different population.

According to Tolbert et al. (1991, 1992), apoptosis and necrosis are cellular phenomena that indicate genotoxic and cytotoxic effects that are associated, respectively, with initiation and promotion of malignant transformation. Thus, inclusion of these nuclear abnormalities increases the sensitivity of the micronucleus test (Tolbert et al., 1992). In this study, the frequency of these abnormalities did not differ between the sample groups, and this result corroborates the findings of the studies by Hook et al. (2004), Merola et al. (2006) and Yadav and Sharma (2008).

Thus, out of all the endpoints analyzed, broken egg structures were the only one with a significant difference in occurrences between the groups, such that these structures occurred more frequently in group I. Yadav and Sharma (2008) also evaluated the occurrences of these structures in exfoliated cells from the oral mucosa of users and nonusers of mobile phones, but they did not observe any significant difference. It is possible that their result arose through considering the users' annual length of exposure in making up their sample group. Moreover, they did not state what the daily and/or weekly frequency of use of mobile phones was in their groups, which were formed according to the duration of exposure in years. Daily and/or weekly frequency is an important variable in relation to evaluating damage by means of the micronucleus test on exfoliated cells, given that according to Thomas et al. (2009), the micronuclei in these cells reflect recent damage that occurred in the basal cells of the oral epithelium. Such damage is observed 7 to 21 d after it occurs.

According to Nersesyan (2005), the origin and biological significance of the broken egg structures is still not fully understood. In a pioneering study, Tolbert et al. (1992) put forward the idea that their occurrence might be related to genotoxicity. However, in most studies in which occurences of these structures were evaluated, no such association was found (Cerqueira et al., 2004; Cerqueira et al., 2008; Go´mez-Arroyo et al., 2000; Jen et al., 2002; Torres-Bugarı´n et al., 1998).

It has also been proposed that broken egg structures are related to gene amplification (Fenech, 2002; Fenech and Crott, 2002), but the scarcity of reports in the literature investigating an association between gene amplification and exposure to EMF-RF limits the discussion. On the other hand, Nindl et al. (1997) described inhibition of DNA synthesis in Jurkat cells after exposure to EMF. According to Schimke et al. (1986), Hoy et al. (1987) and Shimizu et al. (1998), the process of gene amplification is related to this inhibition.

However, it is possible that the action of the EMF does not make itself felt through inhibition of DNA synthesis but, rather, through transcriptional activation of genes that code for proteins relating to DNA synthesis. The potential for EMF to alter the transcription levels and induce protein synthesis has been pointed out in several studies (Blank and Goodman, 2008; Goodman and Henderson, 1988; Nikolova et al., 2005; Zeng et al., 2006).

On the other hand, Nersesyan (2005) suggested that explaining broken egg structures as a product from the process of gene amplification might be more reasonable in relation to situations in which such structures originate in lymphocytes. Moreover, in the cases of exfoliated cells, these structures are perhaps also products of degenerative cellular processes. Thus, additional studies are needed to clarify the biological significance and origin of these structures.

In the light of these results, unless a hypothesis that broken egg structures are markers for genetic damage is confirmed, increased intensity (hours per week) of exposure to EMF-RF emitted by mobile phones is not associated with genotoxic and/or cytotoxic effects. However, there is a need to investigate the relationship between exposure to EMF and gene amplification, thereby contributing toward evaluating the effects of EMF on cells.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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