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Is mobile phone radiation genotoxic? An analysis of micronucleus frequency in exfoliated buccal cells

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ABSTRACT

Electromagnetic fields (EMF) are classified as "possibly carcinogenic" by the International Agency for Research on Cancer (IARC). Some publications have reported associations between EMF exposure and DNA damage, but many other studies contradict such findings. Cytomorphological changes, such as micronuclei (MN), indicative of genomic damage, are biomarkers of genotoxicity. To test whether mobile phone-associated EMF exposure affects the MN frequency in exfoliated buccal cells, we obtained cells smears from the left and right inner cheeks of healthy mobile phone users, aged 18–30 (n = 86), who also completed a characterization survey. MN frequencies were tested for potential confounding factors and for duration of phone use and preferential side of mobile phone use.

No relationship was observed between MN frequency and duration of mobile phone use in daily calls. Cells ipsilateral to mobile phone use did not present a statistically significantly higher MN frequency, compared to cells contralateral to exposure. A highly statistically significant ($p < 0.0001$) increase in MN frequency was found in subjects reporting regular exposure to genotoxic agents. Therefore, our results suggest that mobile phone-associated EMF do not to induce MN formation in buccal cells at the observed exposure levels.

1. Introduction

The biological consequences of exposure to radiofrequencies remain controversial [1,2]. Decades of research [3,4] have yielded contradictory results: studies have reported causal relationships between exposure to radiofrequency electromagnetic fields (RF-EMF) and increased risk of acoustic neuroma [2,5], glioma [6,7], meningioma [8,9], infertility [6], deafness [10], and metabolic changes in brain tissue [9], but others have failed to reproduce these results [11–16]. In light of this uncertainty, the International Agency for Research on Cancer (IARC) classified RF-EMF as "possibly carcinogenic" [17] and consensus on the issue still eludes the scientific community [18–20]. However, with many people now carrying their own RF-EMF sources – mobile phones [21] – clearer results are needed [22,23].

RF ranges from 100 kHz–300 GHz and generates electromagnetic fields that may affect living cells via thermal or non-thermal mechanisms [24,25], although usually only at very high exposure levels [26]. DNA damage may lead to cell senescence, cell death, or malfunction [27,28]. DNA damage caused by genotoxicants [29] can be detected by several methods, including cytological observation of morphologic changes [29,30], such as micronuclei (MN): chromatin fragments resulting from chromosome breakage [31–33], which are also biomarkers of environmental genotoxicity and cancer risk [34–38]. MN form spontaneously at a reported rate of 0.74‰ (95% CI 0.52–1.05) [31]. MN assessments (*e.g.*, using Feulgen's histochemical stain [39]) can detect increased MN frequencies and represent a standardized biomarker of genotoxicity and chromosome damage [32,34,35,40,41]. The oral mucosa is a popular matrix for MN frequency assessments [42–44], due to its rapid turnover (7–21 d [45,46]), minimal invasiveness, and high representation of epithelial tissue, where 90% of cancers arise [36,47–49]. Oral mucosa MN test procedures have been described by the International Collaborative Project on

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Micronucleus Frequency in Human Populations (HUMN) [50], particularly in the publication "The Human MicroNucleus project on eXfoliated buccal cells" (HUMNXL) [31], which is the basis of the methods used in the present study.

Since exposure should be highest in the adjacent oral epithelium [36,44,51], we tested MN frequencies in the right and left inner cheeks [52]. EMF strength decreases quickly with distance to the source (transmitter) [53], so we hypothesized that mobile phone-generated EMF would affect proximate cells to a greater extent. To test this hypothesis, we compared oral mucosa MN frequencies on the ipsilateral (*i.e.*, the side on which the user holds the phone during a call) vs. contralateral side.

2. Materials and methods

2.1. Subjects

This study was approved by the Scientific Division of Histocellular Pathology of the Lisbon School of Health Technology. Healthy volunteers, 18–30 y, studying or working in the Lisbon metropolitan area, were enrolled as study subjects (total, 86), using a convenience (non-probabilistic) sampling method. All participants accepted the terms of the informed consent, allowing the collection of samples from the oral mucosa, and completed a survey on demographic data (gender, age, place of birth), social and environmental background (occupation, duration and recent changes of occupation, residency in proximity to airports or heliports, tobacco and alcohol consumption, meat, fish, fruit and vegetable consumption, vitamin and non-vitamin supplementation, family history of cancer, chronic medication, self-reported risk factors); and EMF exposure (years of mobile phone use, daily use in minutes, preferential side of use (ipsilateral) and iatrogenic exposure over the past 21 days). Participants were also asked about use of headsets or loudspeakers, to enable estimation of daily call duration considering only those calls in which the phone was placed directly next to the head.

2.2. Sample collection

Two separate samples were collected from each subject, one from each inner cheek (right and left). Every collection was made by a trained operator using one sterile cytological spiral endobrush (EndoBrush, Biogyn, Mirandola, Italy) per sample. Each endobrush was then smeared on a slide previously labelled with the subject's identification number and side of collection ("R"/"L"). Smears were fixed with Merckofix® within 10 s of collection, left to dry for a minimum of 24 h, and kept in closed storage boxes until staining.

2.3. Micronucleus test

Slides were stained according to Feulgen's method [39] and air-dried before mounting with synthetic medium. 1000 epithelial cells per slide (2000 cells per subject) were scored by a single trained observer on a Leica DM500 microscope, at 1000× amplification with immersion oil. Scoring criteria were adapted from the most relevant publications [54–56].

2.4. Statistical analysis

An Excel® spreadsheet was used as database for survey responses and cell and MN counts, and then converted to comma-separated values for statistical analysis using the RStudio© software, version 2.1. A Shapiro-Wilk test of the dependent variable (total MN frequency in 2000 cells) showed a non-normal distribution, which led to the selection of the Wilcoxon and Kruskal-Wallis non-parametric tests for further analysis [57–60]. Recommendations for the buccal micronucleus cytome assay were adopted and a significance level of 5% ($p = 0.05$) was set for both tests [54]. All study factors except "side of use" were plotted against MN frequency in 2000 cells using an adequate test (Wilcoxon test for factors with two groups and Kruskal-Wallis test for factors with three or more groups). Side of use was plotted separately against MN frequency in each right and left 1000 cells.

3. Results

The mean age of the study population was 22.6 y (± 2.34, range 18–30) and the male-to-female ratio was 1.15:1. Mean history of mobile phone use in years was 11.4 (± 2.36; range 4–18), with a mean duration of daily calls in minutes of 21.8 (± 18.66; range 2–112).). Nearly 85% of subjects (n = 73) reported the right side as their preferential side of use; about 7% (n = 6) reported the left side and 8% (n = 7) could not specify a preferential side.

Regarding the results of the genotoxicity biomarker in study, MN mean was 2.02 (± 1.65; range 0–7) per 2000 cells. Concerning gender, means were: males (n = 46): 1.74 (± 1.51) MN; females (n = 40): 2.35 (± 1.76) MN; the difference was not statistically significant (Wilcoxon test, $p = 0.0998$).

Subjects were sub-divided into three age groups, for which mean MN frequencies per 2000 cells were: 18–21 years old (n = 30), 2.60 (± 2.04); 22–25 (n = 46), 1.69 (± 1.30); 26–30 (n = 10), 1.80 (± 1.48). No statistically significant relation was found between age groups and mean MN frequency (Kruskal-Wallis test, $p = 0.1921$).

No associations were found between mean MN and the variables assessed in the questionnaire: occupation, alcohol consumption, meat, fish, vegetable and fruit consumption, dietary supplementation, iatrogenic exposure, proximity of residence to an airport or heliport, family history of cancer, chronic medication, and self-reported risk factors (Wilcoxon test, $p > 0.05$; Kruskal-Wallis test, $p > 0.05$ for all parameters). Tobacco consumption (smokers = 20, non-smokers = 66), although a stressor notably associated with increased MN incidence, was also unrelated to the biomarker frequency in the study population (Wilcoxon test, $p = 0.4550$), as discussed later.

A number of subjects reported occupations requiring daily contact with substances with known genotoxic properties, and a statistical test was conducted to verify if such exposure affected MN incidence. From this analysis, a statistically significant (Wilcoxon test, $p = 0.000015$) increase in MN frequency per 2000 cells was observed in subjects reporting this exposure, with mean MN frequency = 3.60 ± 1.73 (reported exposure, n = 18) versus 1.60 ± 1.36 (no reported exposure, n = 68). Smokers were not included in the subset of subjects with reported exposure because no smokers in our population reported consumption of more than 40 cigarettes/day, the threshold above which increased MN frequencies in the oral mucosa are typically observed [31].

Subjects were also grouped into year intervals of mobile phone use for which the mean MN frequencies per 2000 cells were as follows: 9 y or less (n = 9), 2.67 (± 1.80); 9–13 y (n = 61), 1.92 (± 1.72), and > 13 y (n = 16), 2.06 (± 1.29). These differences were not found to be statistically significant (Kruskal-Wallis test, $p = 0.3024$).

To analyse the effect of daily exposure to mobile phone EMF, subjects were sub-divided into quartiles of exposure in min per day (Fig. 1), with the following observations for MN frequency, from the first to the fourth quartile: first (n = 22), 1.54 (± 1.30); second (n = 21), 2.19 (± 1.78); third (n = 21), 2.23 (± 1.83); fourth (n = 22), 2.13 (± 1.67); the differences were not statistically significant (Kruskal-Wallis test, $p = 0.6287$).

On the survey, 79 of 86 subjects specified a preferential side of mobile phone use (either right or left), designated as the ipsilateral side. (The remaining seven subjects did not express a preferential side of use and were excluded from the laterality analysis). For the 79 subjects, mean MN totals on the ipsilateral and contralateral (opposite) sides were compared. Side of mobile phone use did not show an association with MN frequency per 1000 cells (Fig. 2); mean MN frequencies were