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Quantitative analysis of nuclear morphological alterations in oral mucosal epithelial cells exposed to electromagnetic radiation from mobile devices

Enni Halimatussadiyah Pakpahan[1*], Adi Hartono[2]

[1]Clinical Pharmacy Study Program, Faculty of Medicine, Dentistry & Health Sciences, Universitas Prima Indonesia, Jl. Sampul No. 3, Sei Putih Bar, Medan Petisah District, Medan City, North Sumatra, 20118, Indonesia

[2]Biology Education Study Program, Department of Science and Mathematics Education, Faculty of Teacher Training and Education, Universitas Samudra, Jl, Prof. Dr. Syarief Thayeb, Meurandeh, District, Langsa Lama, Langsa, 24416, Indonesia

*corresponding author: ennihalimatussadiyahpakpahan@unprimdn.ac.id

ABSTRACT

Electromagnetic wave (EMW) radiation from mobile devices has raised public health concerns due to its potential biological effects on rapidly regenerating tissues such as the oral mucosa. This study quantitatively analyzed nuclear morphological alterations in oral mucosal epithelial cells following controlled EMW exposure. A quasi-experimental pretest–posttest control group design was conducted with 30 healthy participants aged 18–25 years, randomly assigned to control (n = 15) and treatment (n = 15) groups. The treatment group was exposed to EMW at 1800 MHz for 60 minutes per day over 14 consecutive days, using an active smartphone placed 1 cm from the right cheek during a simulated voice-call condition. The control group received no direct exposure. Buccal epithelial cells were collected via oral swab, fixed in Carnoy's solution, stained with hematoxylin–eosin, and observed under a light microscope at 400× magnification. Quantitative assessment focused on three types of nuclear abnormalities: pyknosis, karyorrhexis, and karyolysis. The Independent Samples t-test showed a significantly higher mean number of nuclear abnormalities in the exposed group than in the control group (12.47 ± 3.15 vs. 4.86 ± 1.92 cells/field; $p < 0.001$). Pyknosis was the most frequent alteration (58.3%), followed by karyorrhexis (26.7%) and karyolysis (15.0%). All procedures were approved by the institutional ethics committee. In conclusion, short-term exposure to 1800 MHz EMW from mobile devices under controlled conditions can induce measurable degenerative nuclear changes in oral epithelial cells, suggesting cellular susceptibility to non-ionizing radiation at the cytomorphological level.

Keywords: Electromagnetic radiation, light microscopy, oral mucosal epithelial cells, quantitative morphological analysis, smartphone exposure

INTRODUCTION

The global increase in smartphone use has made these devices an inseparable part of daily life, particularly among young adults. Smartphones emit non-ionizing radiofrequency electromagnetic fields (RF-EMF) that are often positioned near the head and oral cavity during use. Although RF-EMF is categorized as non-ionizing, chronic exposure has been reported to trigger various cellular and molecular responses, making its biological implications a continuing subject of scientific debate (Gulati et al., 2020; Kundi et al., 2024; Massaro et al., 2022). The oral mucosa, characterized by a high cell turnover rate, is anatomically positioned to receive direct exposure from these emissions, thereby representing a sensitive target tissue for evaluating potential cytomorphological changes (Ye et al., 2025).

Previous studies examining RF-EMF effects on genotoxic and cytotoxic biomarkers in oral epithelial cells have produced inconsistent results. Some investigations have reported an increased frequency of micronuclei or nuclear abnormalities among heavy mobile phone users (Kundi et al., 2024; Revanth et al., 2021), whereas

others found no evidence of acute chromosomal damage but identified cytotoxic alterations such as pyknosis and karyorrhexis (Fenech et al., 2023; Kundi et al., 2024). Methodological advances, such as the Buccal Micronucleus Cytome (BMCyt) assay and liquid-based cytology, have improved the detection sensitivity for nuclear abnormalities (Ghandehari et al., 2021; Santoso et al., 2025). Nevertheless, the diversity of study designs—many of which are cross-sectional or observational—limits causal inference and the interpretation of dose–response relationships (Fenech et al., 2024; Gouseti et al., 2023). Furthermore, few human studies have simultaneously quantified multiple nuclear degeneration markers—pyknosis, karyorrhexis, and karyolysis—under well-controlled exposure conditions (Kadeh et al., 2023; Kulikov & Korolev, 2025; Torabinia et al., 2024).

This research addresses these gaps by applying a quasi-experimental pretest–posttest control group design involving 30 participants with controlled exposure duration and intensity. Compared with previous observational or semi-controlled studies, this design offers methodological advancement through random group assignment, standardized RF-EMF measurements, and quantitative evaluation using hematoxylin–eosin staining observed under light microscopy (Sangle et al., 2023; Wouters et al., 2016). Such an approach enhances internal validity and allows a clearer causal interpretation of the relationship between EMF exposure and nuclear morphological alterations.

Accordingly, the present study aims to quantitatively analyze nuclear morphological changes in oral mucosal epithelial cells induced by 1800 MHz RF-EMF exposure from smartphones. The specific objectives are to (1) compare the frequency of nuclear abnormalities between treatment and control groups, and (2) evaluate the predominance of specific nuclear degeneration types (pyknosis, karyorrhexis, and karyolysis).

The working hypothesis of this study is that controlled exposure to 1800 MHz RF-EMF from smartphones for 60 minutes daily over 14 days significantly increases the frequency of nuclear abnormalities in oral mucosal epithelial cells compared with unexposed controls. This hypothesis provides the framework for interpreting whether the experimental findings confirm, refute, or partially support the anticipated cytomorphological effects of non-ionizing electromagnetic radiation.

METHOD

This study adopted a quasi-experimental pretest–posttest control group design to examine the effects of electromagnetic radiation from mobile devices on nuclear morphology in oral mucosal epithelial cells. The design allows intra- and intergroup comparisons to strengthen causal inference and control for confounding variables (Campbell & Stanley, 2015; Dkhar et al., 2025; Mosibo et al., 2024).

Thirty healthy, non-smoking, and non-alcohol-consuming university students aged 18–25 years participated voluntarily after providing written informed consent. The study protocol was approved by the Institutional Ethics Committee of Universitas Prima Indonesia. Exclusion criteria included oral mucosal diseases, mucosal injury within the past month, current medication with genotoxic or cytotoxic effects, and prior exposure to ionizing radiation or chemical mutagens within six months.

Sample size was calculated through power analysis ($\alpha = 0.05$; power = 0.8; effect size $d = 0.8$, as recommended by Cohen, 1992), yielding a minimum of 30 participants (15 per group). Participants were randomly assigned to treatment and control groups using simple randomization.

The treatment group was exposed to radiofrequency electromagnetic waves (RF-EMW) emitted by a smartphone (Brand: Samsung Galaxy A14, Model SM-A145F, FCC ID: A3LSMA145F) operating at 1800 MHz (4G LTE

band) for 60 minutes daily over 14 consecutive days. During exposure, the device was positioned 1 cm from the right cheek under simulated voice-call conditions. The emitted field intensity was verified using a calibrated EMF meter (TES-593, TES Electrical Electronic Corp., Taiwan; detection range 0.1–2000 $\mu W/cm^2$; frequency sensitivity 900–1800 MHz). The mean measured power density during exposure was recorded at 1.65 ± 0.12 mW/cm^2. The control group was not directly exposed; participants were instructed to avoid active phone calls during the study period, and environmental EMF in the control room was continuously monitored to ensure background levels < 0.05 mW/cm^2. All exposures were conducted in a temperature-controlled room (25 ± 1°C, 45–55% humidity).

Buccal epithelial cells were collected 24 hours before the first exposure (pretest) and 24 hours after the last exposure (posttest). Sampling was conducted between 08:00–10:00 a.m. to minimize circadian effects (Tolbert et al., 1992). After rinsing the mouth with distilled water for 30 seconds, the inner buccal mucosa was gently scraped using a sterile cytobrush (Gynobrush, HVD Life Sciences, Cat. No. 10.202). Samples were immediately fixed in freshly prepared Carnoy's solution (absolute ethanol: glacial acetic acid = 3:1) for 30 minutes.

Slides were stained exclusively with hematoxylin–eosin (HE) for the identification of nuclear morphology, as it provides optimal contrast for pyknosis, karyorrhexis, and karyolysis visualization (Schmid, 1975). The Feulgen method was not applied in this study. Each slide was air-dried, dehydrated through graded ethanol, cleared in xylene, and mounted with DPX medium (Merck, Cat. No. 100579). Observation was performed under a light microscope (Olympus CX23, Japan) at 400× magnification by two independent, blinded observers.

A total of 1000 well-preserved epithelial cells per participant were scored according to the criteria by Fenech et al. (2023). Nuclear abnormalities were classified into pyknosis, karyorrhexis, and karyolysis. Inter-observer agreement was verified (Cohen's $\kappa = 0.82$).

Statistical analyses were conducted using IBM SPSS Statistics v28. Normality was tested using the Shapiro–Wilk test. Intergroup comparisons were made using the independent samples t-test for normally distributed data and the Mann–Whitney U test for non-normal data. Correlations between EMF intensity and nuclear abnormality frequency were analyzed using Pearson's or Spearman's test depending on data distribution. Statistical significance was set at $p < 0.05$ (Field, 2018).

RESULTS AND DISCUSSION

The study was completed by a total of 30 participants, with 15 in the control group and 15 in the treatment group. All individuals involved in the study were young, non-smoking students between the ages of 18 and 25, and were in good health. As delineated in Table 1, both cohorts exhibited similar baseline characteristics, with no noteworthy disparities in age, gender distribution, or oral hygiene index ($p > 0.05$). This uniformity affirms that any disparities observed in the post-test results can be attributed to the controlled electromagnetic exposure rather than existing variability.

Table 1. Demographic and Baseline Characteristics of Participants (n = 30)

Variable	Control (n = 15)	Treatment (n = 15)	p-value
Age (years, mean ± SD)	21.4 ± 1.9	21.6 ± 2.0	0.84
Gender (M/F)	6/9	7/8	0.71
Daily phone use (hours)	≤ 0.5	2.0 ± 0.3	<0.001
Distance from device (cm)	>50	1 ± 0.2	<0.001
Oral hygiene index	0.92 ± 0.14	0.95 ± 0.12	0.56

Table 2 displays the comparison of nuclear abnormalities between groups. Following 14 consecutive days of 1800 MHz RF-EMF exposure for 60 minutes per day, the treatment group demonstrated a significantly higher frequency of

nuclear abnormalities in comparison to the control group ($p < 0.001$). In particular, the average values for pyknosis, karyorrhexis, and karyolysis were notably increased, resulting in a total abnormality frequency of 12.47 ± 3.15 per 1000 cells in the treatment group as opposed to 4.86 ± 1.92 in the control group.

Table 2. Frequency of Nuclear Abnormalities per 1000 Buccal Epithelial Cells

Nuclear Abnormality	Control (mean ± SD)	Treatment (mean ± SD)	p-value
Pyknosis	2.81 ± 0.96	7.26 ± 1.43	<0.001
Karyorrhexis	1.11 ± 0.54	3.11 ± 0.85	<0.001
Karyolysis	0.94 ± 0.48	2.10 ± 0.71	0.002
Total abnormalities	4.86 ± 1.92	12.47 ± 3.15	<0.001

The representative photomicrographs of buccal epithelial cells that have been stained with hematoxylin–eosin are depicted in Figure 1. Panels (a)–(d) depict the successive nuclear transformations that have been observed: normal nucleus (a), pyknosis (b), karyorrhexis (c), and karyolysis (d). The morphological changes, observed primarily in the treatment group, are indicative of degenerative and apoptotic processes, aligning with the quantitative data presented in Table 2.

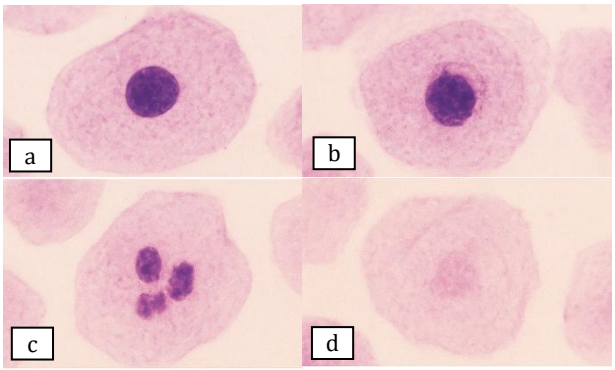

Figure 1. Representative photomicrographs of buccal epithelial cells (HE staining, 400×): (a) normal nucleus (control); (b) pyknosis; (c) karyorrhexis; and (d) karyolysis. Scale bar = 10 µm.

According to the data presented in Figure 2, the linear regression analysis revealed a significant positive association between the length of daily exposure and the total number of nuclear abnormalities ($r = 0.712$; $p < 0.001$; $R^2 = 0.56$). The inclination of the regression line suggests that an increase of 30 minutes in daily exposure led to an estimated 1.5 additional nuclear abnormalities per 1000 cells. This pattern provides clear evidence of a dose-dependent impact of electromagnetic radiation on cellular shape, in line with the results presented by Kundi et al. (2024) and Massaro et al. (2024).

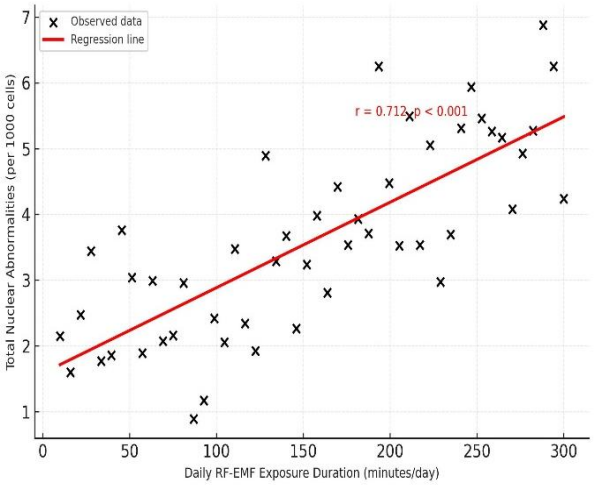

Figure 2. Scatter plot showing the relationship between daily RF-EMF exposure duration (minutes/day) and total nuclear abnormalities per 1000 cells ($r = 0.712$, $p < 0.001$).

The significant rise in pyknosis, karyorrhexis, and karyolysis (as demonstrated in Table 2 and Figure 1) suggests that exposure to RF-EMF induces cytotoxic effects rather than solely genotoxic ones. From a mechanistic standpoint, these changes are linked with the production of reactive oxygen species (ROS), impairment of mitochondrial function, and the occurrence of DNA strand breaks, resulting in chromatin condensation and nuclear fragmentation (Fenech et al., 2023; Wang et al., 2024; Zhao et al., 2022). This mechanism is additionally reinforced by empirical research that indicated heightened oxidative biomarkers as a result of prolonged exposure to radiofrequency radiation (Khalil et al., 2020; Kumar et al., 2021; Tong et al., 2025). The

occurrence of binucleated cells, while not the primary observation, indicates a disruption in cytokinesis—a phenomenon identified by Thomas & Fenech (2009) as an early indication of interference with the mitotic spindle under conditions of oxidative stress.

The data displayed in Table 2 align with previous research, exemplified by Kadeh et al. (2023) and Revanth et al. (2021) also documented an increase in nuclear abnormalities in individuals who frequently use smartphones. Nevertheless, the degree of change in the current study was greater, possibly as a result of the controlled frequency and duration of exposure. In contrast to observational designs, the quasi-experimental arrangement in this case, when combined with pretest-posttest comparison, permits a more robust inference of causality.

The results of the normality testing confirmed that the parametric assumptions were satisfied. Therefore, independent t-tests were utilized, as detailed in Table 2. In order to reduce the occurrence of false positives, the Bonferroni correction was utilized, resulting in an adjusted alpha level of 0.0125. The effect size (Cohen's d = 1.8) demonstrates a significant treatment effect, and the inter-observer reliability ($\kappa = 0.82$) verifies the consistency in scoring nuclear features. The scatter distribution pattern depicted in Figure 2 serves to confirm the strength and reliability of the dose-response relationship (Li et al., 2025; Reimann, 2020; Nguyen, 2022).

The combination of quantitative data (shown in Table 2), visual representation (as depicted in Figure 1), and analytical results (presented in Figure 2) collectively indicates that controlled 1800 MHz RF-EMF exposure at close proximity causes notable cytomorphological alterations in oral epithelial cells. The observed cellular response is significantly influenced by behavioral factors, specifically the proximity of the device and the length of exposure. The results align with the guidelines set forth by ICNIRP (2020), which underscore the importance of reducing exposure time and adhering to safe usage distances. However, the research is constrained by a limited number of participants and the lack of biochemical markers for oxidative stress. Future studies should encompass SAR-based dosimetry, molecular assays such as γ-H2AX, comet assay, and 8-OHdG, as well as larger multicenter trials for the purpose of validation.

The data presented in Table 2 and depicted in Figures 1–2 collectively demonstrate compelling experimental proof that repeated, short-term exposure to 1800 MHz RF-EMF can cause degenerative nuclear alterations in oral epithelial cells in a manner that is dependent on the dose. The results of this study provide evidence for the biological plausibility of oxidative stress induced by RF-EMF, and emphasize the importance of using mobile devices prudently to decrease the long-term risk of cellular damage.

CONCLUSION

This study demonstrated that controlled exposure to 1800 MHz radiofrequency electromagnetic fields (RF-EMF) from mobile devices for 60 minutes per day over 14 consecutive days induced measurable cytomorphological alterations—specifically increased frequencies of pyknosis, karyorrhexis, and karyolysis—in oral mucosal epithelial cells. These findings provide quantitative evidence that short-term, close-range RF-EMF exposure can elicit degenerative nuclear changes consistent with cytotoxic rather than genotoxic mechanisms. While the results indicate a dose-dependent trend between exposure duration and nuclear abnormalities, the conclusions are limited to the specific experimental conditions tested.

Future research should expand upon these findings by employing larger and more diverse populations, longer exposure durations, and comprehensive dosimetric analysis such as specific absorption rate (SAR) modeling. Integrating molecular assays—such as comet

assays, γ-H2AX detection, and oxidative stress biomarkers (MDA, 8-OHdG, SOD)—would help elucidate the underlying mechanisms linking RF-EMF exposure to nuclear damage. Moreover, comparative studies across different device technologies (e.g., 4G vs. 5G) and behavioral patterns could provide a more precise understanding of real-world exposure impacts and contribute to the formulation of evidence-based public health recommendations.

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