



Comment

# Comment on Pop et al. Early Diagnosis of Oral Mucosal Alterations in Smokers and E-Cigarette Users Based on Micronuclei Count: A Cross-Sectional Study among Dental Students. *Int. J. Environ. Res. Public Health* 2021, 18, 13246

Wilton Mitsunari Takeshita <sup>1</sup> and Daniel Araki Ribeiro <sup>2,\*</sup>

<sup>1</sup> Department of Dentistry, Federal University of Sergipe, UFS, Aracaju 49060-108, SE, Brazil; wmtakeshita2@gmail.com

<sup>2</sup> Department of Biosciences, Institute of Health and Society, Federal University of São Paulo, UNIFESP, Santos 11015-020, SP, Brazil

\* Correspondence: daribeiro@unifesp.br; Tel.: +55-1332290156



**Citation:** Takeshita, W.M.; Ribeiro, D.A. Comment on Pop et al. Early Diagnosis of Oral Mucosal Alterations in Smokers and E-Cigarette Users Based on Micronuclei Count: A Cross-Sectional Study among Dental Students. *Int. J. Environ. Res. Public Health* 2021, 18, 13246. *Int. J. Environ. Res. Public Health* 2022, 19, 3713. <https://doi.org/10.3390/ijerph19063713>

**Academic Editors:** E. Melinda Mahabee-Gittens and Ashley L. Merianos

Received: 6 January 2022

Accepted: 9 March 2022

Published: 21 March 2022

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

We read the manuscript recently published in *International Journal of Environmental Research and Public Health* titled “Early diagnosis of oral mucosal alterations in smokers and e-cigarette users based on micronuclei count: a cross-sectional study among dental students” by Pop et al. [1] with much interest. In the study, the authors proposed the application of a micronucleus assay as a suitable tool for detecting cytogenetic damage in buccal cells. In the study, the authors found a high number of micronuclei in smokers and e-cigarette users when compared to non-smokers (control group). However, some theoretical concepts and guidelines are described for the correct understanding of the manuscript.

In the manuscript, it was written that all specimens were stained with Papanicolaou stain (Pap stain). In the Discussion, it was stated that “Therefore, based on the acceptable accuracy regarding MN evaluation, Pap stain may be used for routine screening and samples with potential abnormalities can be further processed with DNA-specific stains”. It is important to highlight that Pap stain is not the best possible option for micronucleus testing because there are structures in the cytoplasm of buccal cells that are identical to micronuclei, such as, for example, cytoplasmic granules or inflammatory cells, and it is therefore, impossible to identify it with accuracy [2]. Moreover, a figure in the range from 0.3 to 1.7/1000 was considered to be correct in considering the spontaneous micronucleus incidence in buccal cells by the micronucleus assay guidelines [2]. The data presented in the manuscript for the control group in MC count and MNC count were  $1.95 \pm 1.05$  and  $1.4 \pm 0.68$ , respectively. Certainly, these results were related to false-positive data.

As usual, all Tables and Figures should be self-explanatory, i.e., they should provide clear presentations and syntheses of the results found. Herein, what does Groups A–C, MN and MNC presented in Tables 1–3 and Figure 3 mean? This information is present only in the Results. In Table 2 and Figure 2, the authors indicated the analysis of micronucleus frequency by means of the total number of micronuclei (MN) and total micronucleated cells (MNC). What is the biological and clinical relevance of the approach? Taking into consideration the micronucleus assay guidelines, there is not any significance linked to the total number of micronucleus in the any eukaryotic cell.

In Material and Methods, a total of 1000 buccal cells were counted per volunteer. Following the guidelines proposed by the International Micronucleus Assay Group, the analysis of 2000 cells per person has been established [2]. Certainly, the approach would dramatically improve the quality of the study data.

Interestingly, the Discussion starts with the phrase “The aim of this study was to determine whether smokers or e-cigarette users exhibit cytotoxic damage of the buccal mucosa in the absence of any significant clinical manifestation noticeable to the individual,

which could be a false indicator of wellbeing". In our opinion, the statement does not make any sense, since the presence of a micronucleus suggests mutagenic effects as a result of chromosome breakage, and not cytotoxicity.

Finally, it is worth mentioning that Tolbert et al. [3] have revealed the presence of metanuclear changes, suggesting the presence of cytotoxicity in buccal cells, such as for example, karyorrhexis, pyknosis and karyolysis. The approach is valid and necessary because cytotoxicity interferes with the amount of micronuclei. If cytotoxicity is positive, the micronucleated cells are subtracted at the expense of cellular death. Particularly, this is clearly seen in the oral cells from chronic smokers [4–6].

We assumed that such comments are useful for the correct understanding of the manuscript validating the micronucleus assay in smokers and e-cigarette users as a suitable tool using buccal cells.

**Author Contributions:** W.M.T. and D.A.R. wrote the letter. All authors have read and agreed to the published version of the manuscript.

**Funding:** D.A.R. is a recipient of CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, grant number #001) productivity fellowship.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Pop, A.M.; Coroş, R.; Stoica, A.M.; Monea, M. Early Diagnosis of Oral Mucosal Alterations in Smokers and E-Cigarette Users Based on Micronuclei Count: A Cross-Sectional Study among Dental Students. *Int. J. Environ. Res. Public Health* **2021**, *18*, 13246. [[CrossRef](#)] [[PubMed](#)]
2. Bonassi, S.; Coskun, E.; Ceppi, M.; Lando, C.; Bolognesi, C.; Burgaz, S.; Holland, N.; Kirsh-Volders, M.; Knasmueller, S.; Zeiger, E.; et al. The HUMAN MicroNucleus project on exfoliated buccal cells (HUMN(XL)): The role of life-style, host factors, occupational exposures, health status, and assay protocol. *Mutat. Res.* **2011**, *728*, 88–97. [[CrossRef](#)] [[PubMed](#)]
3. Tolbert, P.E.; Shy, C.M.; Allen, J.W. Micronuclei and other nuclear anomalies in buccal smears: Methods development. *Mutat. Res.* **1992**, *271*, 69–77. [[CrossRef](#)]
4. Metgud, R.; Neelesh, B.T. Effect of staining procedures on the results of micronucleus assay in the exfoliated buccal mucosal cells of smokers and nonsmokers: A pilot study. *J. Cancer Res. Ther.* **2018**, *14*, 372–376. [[PubMed](#)]
5. Nersesyan, A.; Kundi, M.; Atefie, K.; Schulte-Hermann, R.; Knasmüller, S. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. *Cancer Epidemiol. Biomark. Prev.* **2006**, *15*, 1835–1840. [[CrossRef](#)] [[PubMed](#)]
6. da Silva, V.H.P.; de Luna Antonio, R.; Pompeia, S.; Ribeiro, D.A. Cytogenetic biomonitoring in buccal mucosa cells from young smokers. *Acta Cytol.* **2015**, *59*, 474–478. [[CrossRef](#)] [[PubMed](#)]