LETTER TO THE EDITOR

IS MICRONUCLEUS ASSAY SUITABLE FOR BIOMONITORING OF CHILDREN UNDERGOING TOOTH RESTORATION? SOME CONCEPTS AND DEFINITIONS

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I have read the recent paper published in the Central European Journal of Public Health titled "In vivo assessment of genotoxicity in buccal cells of children undergoing tooth restoration" by Gavić et al. (1) with much enthusiasm. In the manuscript, the authors found high frequencies of micronuclei and binucleated cells in children undergoing tooth restoration. Particularly, mutagenicity was detected in oral cells from children continuously exposed to Ketac Molar, Twinky Star and Ionofil Molar. However, some concepts and definitions must be clearly explained for the correct understanding of the manuscript.

In Materials and Methods, it was mentioned that "a buccal swab was taken by gently brushing the gingival area along the glass-ionomer or compomer restoration with an interdental brush and applied to encoded microscopic slides pre-warmed at 37 °C". However, it was written several times that micronucleus assay was performed in buccal cells. What was the cell type evaluated in this study? This needs clarification.

It is important to stress that Giemsa stain should not be applied when performing the micronucleus assay since it is not specific for nucleic acids (2). In light of the lack of DNA specificity of the technique, the micronucleus identification is very hard due to the presence of some structures in the cytoplasm of oral cells that are identical to micronucleus, such as bacteria or even inflammatory cells. In addition, a total of 1,000 epithelial cells were evaluated per volunteer. According to the Micronucleus Assay Expert Group, it is widely recommended to evaluate a minimum of 2,000 cells per individual (2). Certainly, to increase the number of cells evaluated would significantly improve the quality of the data, particularly because standard deviations are very high for all groups and periods evaluated in this study.

Unfortunately, the results are confusing and were not properly described in the manuscript. For example, it was written in Results that "As opposed to that, the frequency of karyolysis was statistically significant – higher in samples taken 30 days following restoration ($p < 0.001$) compared to the day 0 but also compared to the day 7 and 90 ($p = 0.020$)". In Discussion, it was stated that "In our research, the significant increase in karyolysis was observed only after 30 days after treatment with Twinky Star". However, Figure 1 does not confirm these findings described by the authors. In the same Figure, it was mentionated "Statistically significant values ($p < 0.05$) between materials in the same time-point of measurement". What does it mean? In my opinion, such comparison does not have any biological significance.

Finally, Tolbert et al. (3) have incorporated some meta-nuclear changes indicative of cellular death (cytotoxicity) for the micronucleus assay in exfoliated cells, such as karyorrhexis, pyknosis and karyolysis. This is very important issue, because cytotoxicity is a confounding factor for mutagenicity (3). If cytotoxicity is increased, the micronucleus frequency automatically decreases, because micronucleated cells are lost due to cellular death. In Figure 2, it was demonstrated zero values for karyorrhexis and pyknosis in some periods evaluated in the study. How is it possible if these cellular changes comprise a normal process of epithelial differentiation?

I hope that such comments are useful for the correct understanding of the paper validating the micronucleus assay as suitable tool for biomonitoring of children undergoing tooth restoration.

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Conflict of Interests

None declared

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