## Letter to the Editor—the Use of Micronucleus Assay on Buccal Mucosa Cells for Risk Assessment: Relevance of Cigarette Smoke and Cytogenotoxicity



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In a recent study published by Alabi and colleagues [1], high levels of some non-essential metals such as Pb, Ni, Cd, and Cr in serum were associated with DNA damage and cytotoxicity of teenage scavenger in Nigeria as depicted by the micronucleus assay on buccal mucosa cells. However, we identified some questions that should be addressed properly for better understanding the paper.

In Material and Methods, Giemsa was used for staining slides. Nevertheless, the technique is not suitable for this purpose since it is not specific for nucleic acids. This inevitably leads to false positive results due to the identification of cell structures that remember micronucleus, such as keratohyalin granules or even bacteria [2]. This may explain the high micronucleus frequencies found by the authors (mean of 3.23 micronuclei for negative control group; 168.04 for Alaba group; and 101.14 for positive control). It is important to stress that micronucleus is an uncommon event in the oral mucosa, whose formation resides on dividing cells from the basal layer of oral epithelium, being detected after some days in exfoliated keratinocytes [3].

Another question refers to the positive control (smokers). In Material and Methods, it was stated that "group A (positive control): smears from exfoliated buccal cells were obtained from five cigarettes smokers with more than a year continuous smoking experience. Smoking of cigarette is an established inducer of MN." To support this claim, the authors were able to cite three references, but some of them were wrongly interpreted. For example, the article published by Boherer and colleagues (Reference number 10) did not show significant statistically differences for micronucleated cells on oral cells between smokers and non-smokers [4]. The second paper (Reference number 28) refers to Betel quid/areca nut use and not cigarette smoke [5]. Herein, these ones should not be cited in the manuscript. Anyway, it has been established that increased micronucleus frequency induced on oral mucosa cells by cigarette smoke is strongly dependent on the type of cigarette as well as the number of cigarettes per day [6].

In Table 5 of Alabi et al. [1], the results from pyknosis and karyorthexis show mean of zero values in the negative control group. How is it possible if these metanuclear changes comprise the normal process of epithelial differentiation? In the same Table, the mean frequency of lobbed nuclei from buccal cell is presented. What is the biological significant of this parameter regarding mutagenesis? Moreover, Figure 2d of Alabi et al. [1] does not seem micronucleus since it does not fulfill the same texture and staining intensity as that presented by the main nucleus; Figure 2f is not possible to identify the condensed chromatin (the nucleus is very dark) and Figure 2g of Alabi et al. [1] is not karyorthexis. These require further clarification.

We hope that these comments be useful for better understanding the important article investigating cytogenetic damage on oral mucosa cells in teenage scavengers continuously exposed to waste.

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## **Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** This article did not contain human participants or animals.

Informed Consent For this type of study, formal consent is not required.

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