nuclei in the oral mucosa of crack cocaine users. However, there are certain questions related to data interpretation and accuracy that must be addressed with regard to this study.

First, it is important to stress that Giemsa is an inappropriate technique for detecting micronucleated cells, since it is not specific for staining nucleic acids. The Feulgen–fast green method has been considered the gold standard for this purpose and is highly recommended by the International Human Micronucleus Project on Exfoliated Buccal Cells[2]. Giemsa stain can cause false-positive results due to the presence of keratohyaline granules and/or bacteria, thus the identification of micronuclei will be overestimated[3]. This may explain the high numbers of micronuclei presented in Table 3 (mean of 17.2 micronuclei in the experimental group and 3.8 micronuclei in the control group). Micronucleus is a rare event in the oral mucosa, whose formation arises in the dividing cells in the basal layer of the oral epithelium, with later detection in exfoliated keratinocytes following differentiation[4].

Regarding cytotoxicity, the authors showed increased karyorrhexis and karyolysis in buccal mucosa cells from crack cocaine users. Karyolysis is closely associated with necrosis, whereas karyorrhexis is linked to apoptosis[4]. Independent of the biological phenomenon of cellular death, cytotoxicity participates in the non-genotoxic mechanisms of carcinogenesis, since it interferes with cell proliferation in the eukaryotic cell (promotion stage). Taken as a whole, the results of this study clearly demonstrate that mutagenicity and cytotoxicity are induced by crack cocaine in oral mucosa cells. These findings could explain the higher incidence of oral lesions in crack cocaine users, especially in some regions considered at high risk of oral cancer, such as the floor of the mouth and tongue. Following this rationale, it would be important to compare the frequencies of micronucleus, as well as other nuclear alterations indicative of cytotoxicity, among volunteers with and without oral lesions visible at clinical examination as experimental and control groups, in order to correlate the metanuclear alterations and the frequency of oral lesions. Such information would contribute towards the validation of the micronucleus assay as a putative biomarker for oral diseases in this vulnerable population and others as well.

Finally, the authors report that ''Control individuals with history of street drug use were recruited from public schools or from a list for treatment at the dental school who required an examination before undergoing treatment''. It is well established that illicit drugs are able to induce genotoxic damage[5]. A control group not exposed to known genotoxins would also be interesting in this setting to confirm the cytogenetic damage mediated exclusively by crack cocaine in buccal mucosa cells[6].

We hope that these comments are useful for better understanding the interesting article investigating the relationship between crack cocaine, cytogenetic damage, and oral lesions.

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Competing interests

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Ethical approval

Not applicable.

Patient consent

Not applicable.

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Response to the Letter to the Editor regarding ''Impact of crack cocaine use on the occurrence of oral lesions and micronuclei''

We are grateful for the interest in our study ''Impact of crack cocaine use on the occurrence of oral lesions and micronuclei''[1]. Firstly, regarding the criticism that Giemsa stain can cause false-positive results, we agree that this method may provide an overestimate of the presence of micronuclei. However, any misclassification would be non-differential, as a difference in the overestimation between groups is not plausible. Thus, this type of error would bias the results towards the null hypothesis[2] and therefore does not account for the differences described in our study between crack users and non-users. Moreover, despite its limitations, Giemsa solution has been used to evaluate the presence of micronuclei in different fields of dentistry, with articles published in high-impact journals[3,4].

Another criticism was that ''it would be important to compare the frequencies of micronucleus, as well as other nuclear alterations indicative of cytotoxicity, among volunteers with and without oral lesions visible at clinical examination as experimental and control groups, in order to correlate the metanuclear alterations and the frequency of oral lesions''. We would like to clarify that the aim of our study was to compare the prevalence of fundamental lesions in the oral mucosa and the frequency of cell damage in crack users and controls. Although the topic is interesting, investigating the association between micronuclei and oral lesions constitutes a different objective, other than that proposed in our study. Moreover, as clearly described in the Methods section, the cells were collected with swabs from the right and left sides of the buccal mucosa and not from the buccal lesion of the participants, making it impossible to investigate the suggested association. In our study, no participant with oral lesions exhibited broken-egg nuclei, which also makes it impossible to compare the groups with and without oral lesions.

The authors would also like to correct a typographical error in the final edition of the article and clarify that the group of non-users was composed of individuals *without* a history of street drug use, and not to the contrary, as described previously in other publications reporting on different outcomes in the population[5–7].

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Competing interests

The authors declare no conflicts of interest.

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Patient consent

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