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Cytogenetic Biomonitoring in Buccal Mucosa Cells of Young Smokers

Armen Nersesyan[a], *Gohar Parsadanyan*[b], *Gayane Zalinyan*[c], *Naira Chobanyan*[d]

[a]Institute of Cancer Research, Internal Medicine I, Medical University of Vienna, Vienna, Austria; [b]Yerevan State Medical University, and [c]Yerevan State University, Yerevan, Armenia; [d]DeVry Educational Group, American University of the Caribbean School of Medicine, Chicago, Ill., USA

Dear Editor,

The article by da Silva et al. [1] entitled 'Cytogenetic biomonitoring in buccal mucosa cells from young smokers' was read with great interest since we have published several papers concerning the influence of smoking on the formation of micronuclei (MN) and other nuclear anomalies in smokers [2–4]. The authors of the discussed paper found a strong effect of smoking on MN formation, ranging from 0 in control subjects to 0.7% in smokers. At the same time, the levels of 3 nuclear anomalies other than MN (i.e. pyknosis, karyorrhexis and karyolysis) were not increased significantly in the smokers. Although the aim of the study is of great interest (i.e. the prediction of oral cancer risks by means of buccal MN assay in smokers), the paper unfortunately contains several shortcomings which can confuse the reader.

In our opinion, in studies on the effect of smoking on the genotoxicity and cytotoxicity of buccal cells, more data than presented by the authors are needed, including the exact number of cigarettes smoked per day, exact duration of smoking, pack-years, types of cigarettes consumed, and total weight of tar and nicotine consumed by the smokers (for example, see Nersesyan et al. [3]). The data obtained by the authors are in contrast with our findings [2, 3] in which we showed that the smoking of filtered cigarettes does not induce MN in buccal mucosa cells, but significantly increases the levels of many nuclear anomalies. Only smoking of unfiltered cigarettes was a cause of both increased levels on MN and other nuclear anomalies in buccal mucosa cells. A letter we addressed to the editor concerning this matter is also of interest [4]. It is noteworthy that Bonassi et al. [5] analyzed almost all the data concerning MN studies in buccal cells published before 2011 and found that 'the pattern of genetic damage associated with the number of cigarettes smoked per day shows that only subjects who smoked the most (i.e. ≥40 cigarettes per day) had a significant increase in MN over nonsmokers (FR = 1.37; 95% CI 1.03–1.82)'. Wu et al. [6] also found that the MN level is increased only in heavy smokers (≥20 cigarettes/day; see also Nersesyan [4]). In the study by Pereira da Silva et al. [1] the participants smoked more than 10 cigarettes per day for at least 5 years. Hence, there is a discrepancy with the analyses of Bonassi et al. [5] and some other studies [6–8]. In the latter study, exfoliated mucosal cells obtained from the lip, tongue and floor of the mouth of smokers were evaluated.

It is unclear (in table 1 of their study) if the authors presented the total number of MN or cells with MN (and also if the means are presented as ± SD or ± SE). This is quite a serious shortcoming; moreover, both parameters should be presented for a precise evaluation of the genotoxic effect. Nevertheless, the authors reported that the mean number of cells with MN in the buccal cells of 14 control subjects was equal to zero. Based on our experience in this assay (our first paper was published 25 years ago) we are particularly puzzled because this is hardly possible. Indeed, Bonassi et al. [5] stated that the pooled estimate of the baseline frequency of MN in healthy subjects not knowingly exposed to genotoxic chemical agents or radiation and in the nondiseased (after adjusting for laboratory effects) was 0.74‰. In a meta-analysis of 63 studies carried out by Ceppi et al. [9], the baseline level was 1.098‰. Actually, the baseline level of cells with MN in healthy unexposed subjects is between 0.3 and 1.7‰ [5, 9]. Moreover, in a study by Bolognesi et al. [10], buccal cells obtained from healthy subjects (4 males and 3 females aged 18–26 years) were evaluated by 6 experienced scorers, and the mean number of cells with MN (after the double scoring of slides by each investigator) was 0.48‰ and the total number with MN was 0.54‰. Hence, we think that the authors underestimated MN levels in the control subjects. A possible explanation could be the low number of MN cells, such as the minimum reported by Bonassi et al. [5], i.e. 0.3‰. The authors opted to present MN levels as a percentage, which in this case would be 0.03%. If the authors did not want to present hundredths, the value would be 0.0%, representing a serious mistake in cytogenetic studies. Although this is just speculation, the authors should explain their data in greater detail.

Another serious shortcoming of the paper, in our opinion, is the extremely high level of MN cells in smokers (7‰). In almost all studies on the genotoxicity of tobacco smoke assessed by the MN assay in buccal cells with positive results, the difference between the smokers and the control subjects is approximately a factor of 2.0–2.5 [11–13]. Even if we consider that the level of MN cells in the control subjects was equal to the minimum reported by Bonassi et al. [5], i.e. 0.3‰, the difference is approximately 23-fold! This is not possible because in buccal cells of cancer patients exposed to γ-radiation at a dose of 48 Gy, the level of MN cells is 4-fold higher than in the controls [14], and in those exposed to a dose of 60 Gy it is increased around 25-fold [15]. Tobacco smoke compared to γ-radiation is a very weak genotoxic agent, and clearly the increased MN levels in the buccal cells of smokers should not

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E-Mail karger@karger.com
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0001–5547/16/0000–0000$39.50/0

Correspondence to: Dr. Armen Nersesyan
Institute of Cancer Research, Internal Medicine I, Medical University of Vienna
Borschkegasse 8A
AT–1090 Vienna (Austria)
E-Mail armen.nersesyan@meduniwien.ac.at

be 23-fold. This is why we think Pereira da Silva et al. [1] overestimated the effect of smoking.

Another set of strange data are presented in the second table of their report [1]. Since the authors stated that the results are expressed in percentages, the content of pyknotic cells among buccal cells is 108–110%! Similar concerns relate to the other two anomalies, i.e. karyorrhexis and karyolysis, the content of which is 216 and 173‰, respectively, which is not realistic. Possibly, these data are expressed in pro mille. But even in this case the numbers of pyknotic cells are more than 100, which cannot be! Normally the extent of this anomaly is less than 10‰ in various groups of subjects, e.g. in cancer and Down syndrome patients [10] and healthy subjects [10, 16].

Our final comments concern the protocol of the buccal MN assay, and we question why the authors did not apply the standardized protocol of Thomas et al. [16], which is currently used in the majority of laboratories. Instead they applied the old protocol of Tolbert et al. [17]. It is also unclear whether the criteria used to score the MN were that of Beliën et al. [18] or Pereira et al. [19].

In conclusion, the mode(s) of the carcinogenic action of tobacco smoke (genotoxicity, cytotoxicity, or both) on buccal mucosa cells requires further investigation. Especially the role of nicotine, which has been mentioned by us [3] and also by Pereira da Silva et al. [1], warrants further study. Also, we are sure that Pereira da Silva et al. [1] overestimated the genotoxic effect of smoking. Some clarifications from the authors concerning the unclear points of the paper are certainly warranted.

Disclosure Statement

None of the authors have a conflict of interest in relation to this paper.

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