Response to published letter: "What are the Criteria and Conditions for Performing the Micronucleus Assay in Oral Exfoliated Cells from Waterpipe and Cigarette Smokers?"

Dear Editor,

I recently noticed the printed Letter to Editor entitled "What are the Criteria and Conditions for Performing the Micronucleus Assay in Oral Exfoliated Cells from Waterpipe and Cigarette Smokers?"[1] in International Journal of Preventive Medicine about a previous published article by Jalili and JalayerNaderi in the journal.[2] I was very pleased about the points mentioned about the paper. I believe these notes can improve the obtained results from the studies on micronucleus count of oral buccal mucosa.

According to the findings of the study, there was a significant correlation between the repair index and exposure time to smoke in cigarette smokers (P = 0.03), but the correlation was not significant in the waterpipe smokers (P = 0.55).[2] The details of the data about the number of smoked cigarettes/waterpipe (pack × years = PY) were not mentioned in the published article due to the higher number of pictures and tables. These findings are present here in Tables 1 and 2.

A total of 500 to 4000 cells per samples have been calculated in previous studies. But, in most research studies, a total of 1000 buccal cells have been counted to examine the number of micronucleus. So far, the clinical superiority of 2000 cells compared to 1000 cells of buccal mucosa has not been investigated in investing of nuclear anomalies. This point should be kept in mind; even in studies with 1000 cells, findings have shown an increase in the number of micronuclei in smokers. Nevertheless, it is difficult to obtain a suitable smear with 2000 proper cells without overlapped margins. Obtaining intact cells with a clear margin may require more than one smear. This will probably not be a pleasant experience for patients. It is suggested to examine the preference of counting 2000 cells over 1000 cells in an independent study. Apart from the number of counted cells, other technical interventions such as staining can affect the results. Use of deoxyribonucleic acid (DNA)-specific stains in micronucleus studies have more accurate results compared to non-specific ones. To obtain accurate results, a DNA-specific stain, Feulgen, was used.[2] For this reason, it can be confidently assumed that the results were based on the correct amount of nuclear anomalies (DNA alterations).

Based on Bonassi *et al*.,[3] the effect of counted cells on the number of micronuclei in buccal mucosa is under examination in inter-laboratory projects on micronucleus scoring. The counting of 2000 cells was not emphasized in HUman MicroNucleus project on eXfoLiated buccal cells (HUMNXL) conclusions. It should also be noted that the average number of micro-nuclei was obtained from the total of 60 micronuclei in 30,000 cells.[2] In this way, the contribution of each cell to the incidence of micronuclei will be 0.002, which is a very small number.

The mean number of micronucleus count in controls of Farhadi *et al*.,[4] Akhlaghi *et al*.,[5] Shahsavari *et al*.,[6] and Shahsavari *et al*.[7] studies in Iranian samples was 8.84 ± 4.74, 10.55 ± 6.22, 2.07 ± 0.53, and 27 ± 8.6, respectively. In the studies conducted by Dash *et al*.,[8] Bansal *et al*.,[9] and Balraj *et al*.[10] in India, the mean of micronucleus count in buccal mucosa has been reported to be 4.86 ± 2.4, 4.17 ± 2.99, and 4.35 ± 9.779, respectively.

Table 1: The frequency (mean±SD) of number of smoked cigarettes per year (pack × years=PY) in relation to RI

PY	Repair Index	
	Number of cases	Mean±SD
0-10000	4	3.10±3.45
10001-20000	4	2.66±1.80
20001-30000	1	1
30001-40000	1	2.75
40001-50000	3	3.03±1.9
50001-60000	1	1.33
60001-70000	3	0.96±1.36
70001-80000	0	0
80001-90000	0	0
90001-100000	0	0
100001-200000	8	1.67±4.17
200001-300000	2	2±2.82
300001-400000	1	0.33
400001-500000	2	3.5±4.9

Table 2: The frequency (mean±SD) of number of smoked waterpipes per year (pack × years=PY) in relation to RI

PY	Repair Index	
	Number of cases	Mean±SD
0-100	1	1.18
101-200	10	0.73±0.31
201-300	9	1.63±1.65
301-400	6	1.68±1.83
401-500	1	0.66
501-600	2	0.67±0.19
601-700	0	0
701-800	0	0
801-900	0	0
901-1000	0	0
1001-2000	1	0.27

In this way, it seems that in addition to variables such as lifestyle and technical methods, genetic differences should also be considered in determining the normal range of the micronucleus count in the general population. It is suggested that the importance of racial differences on the number of micronucleus count be examined in future studies.

According to the suggestion "broken eggs and karyorrhexis are impossible to identify", it should be emphasized that the nuclear anomalies were examined based on Tolbert *et al*.[11] The images in the article[2] show these changes properly. The color of nuclear anomalies was similar to that of the main nucleus, and anomaly features are compatible with descriptions of Tolbert *et al*.[11] The repair index (RI) was calculated based on RI = (KL + KR)/(MN + BE). Broken eggs and karyorrhexis counts are necessary to determine the RI.[12] It should be emphasized that the examination of other nuclear changes, including broken egg, karyorrhexis, karyolysis, and even RI has been recently noticed and available information is limited and needs to be completed. In addition, these variables are affected by some factors such as age. Other factors that are still unknown to us may have an effect on these nuclear anomalies. Therefore, these changes need further study. However, it seems that the comparison of results about other nuclear anomalies with the Bonassi *et al*.[3] study is too early and needs more investigation.

It is hoped that sharing comments will improve the proposed protocol for micronucleus investigation for future studies.

Acknowledgment

Nil.

Ethical Consideration

Nil.

Code of Ethics

Nil.

Authors' Contribution

Nil.

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Conflicts of interest

There are no conflicts of interest.

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