E-cig might cause cell damage of oral mucosa

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Objective. The aim of this study was to investigate cytogenetic and cytotoxic damage through the evaluation of micronuclei (MN) and metanuclear anomalies in the oral mucosa of electronic cigarette (e-cig) users.
Study Design. The patients were recruited into 4 groups: e-cig users, smokers, former smokers, and nonsmokers (control). The samples were collected by means of exfoliative cytology of the lateral region of the tongue and the floor of the mouth. The smears obtained were fixed and stained by the Feulgen method for investigation of MN and metanuclear anomalies.
Results. A significant difference was observed for MN frequency only between the smoker and control groups. As for metanuclear anomalies, significant differences were observed: karyolysis between: smokers and control, e-cig and control, as well as former smokers; karyorrhexis: between smoker and control; binucleation: between e-cig and former smoker, as well as control; broken eggs: between e-cig and all other groups; nuclear bud: between e-cig and former smokers, as well as control.
Conclusions. E-cig and alcohol users presented genotoxicity and cytotoxicity in the oral mucosa cells. The use of e-cigs and alcohol by former smokers can cause more damage to the cells of the oral mucosa compared to those who have not used e-cigs. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:435−443)

Electronic cigarettes (e-cigs) were developed in 2003 with the aim of assisting smoking cessation[1] in order to minimize the harm caused by burning tobacco and the many substances present in conventional cigarettes. This electronic system simulates conventional cigarettes by vaporizing nicotine[2] and presents propylene glycol and vegetable glycerin, which serve as humectants and assist in smoke production.[1,3] To date, there is no consensus in the literature regarding the risks and benefits of these devices.[4] Regarding the benefits mentioned in the literature, there is the possibility of significantly reducing toxic substances in comparison with conventional cigarettes,[5,6] besides the possibility of regulating the concentration of nicotine, allowing the gradual reduction of this substance with the possibility of eliminating dependence.[1,3] An important risk factor is the attraction of e-cigs with flavor additives,[2] which encourage the introduction of young people to nicotine dependence.

Exposure to nicotine varies according to the components present in liquids, voltage, or power configurations, and model of e-cig devices.[7] In addition, nicotine addiction is related to pharmacologic, psychosocial, and behavioral factors. Thus, González-Roz et al.[8] suggest that the use of e-cigs presents some social and behavioral patterns similar to the habit of smoking conventional cigarettes. Although some e-cig users are addicted, the levels of dependence appear to be lower than those of smoking patients.[8] Foulds et al.[9] compared individuals' dependence when they used conventional cigarettes and then with the use of e-cigs, and they observed a reduction in the levels of dependence. Therefore, more studies are needed to fill some gaps in the knowledge about e-cigs, such as whether e-cig consumption is less harmful than the consumption of conventional cigarettes and whether smoking cessation through use of e-cigs is safe.

The buccal micronucleus cytome (BMNcyt) assay is a noninvasive approach for evaluating genomic damage, chromosomal instability, and cell death in exfoliated cells of the oral mucosa. It can indicate an increased risk of carcinogenesis and chronic diseases.[10-12] Micronuclei (MN) are the most commonly evaluated and are characterized by rounded structures alongside the main nucleus resulting from the separation of a small nucleus fragment during cell division, they and are important for the

Statement of Clinical Relevance

Tobacco consumption promotes cytotoxic and cytogenetic damage to the oral mucosa epithelial cells. However, this damage decreases with tobacco cessation. The association of e-cigarette and alcohol consumption in former smokers seems to prevent the recovery of this damage.

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biomonitoring of chromosomal aberrations, which may be structural or numerical.[10,13] Binucleation is related to defects in cytokinesis.[10] Broken eggs and nuclear buds are considered as being in the same category of anomalies and indicate chromosomal instability or DNA damage.[10,14] Karyolysis and karyorrhexis are associated with cell death.[10,12,15]

For Tolbert et al.,[15] genotoxicity is more related to the induction of apoptosis in the initiation of carcinogenesis, whereas cytotoxicity is related to the promotion of carcinogenesis. So, the association of evaluation of MN with the other metanuclear anomalies allows an increase in sensitivity in the detection of genotoxicity and cytotoxicity,[16] as they tend to be more prevalent than MN.[15]

In view of the above, it is appropriate to investigate cytogenetic and cytotoxic damage through the evaluation of MN and metanuclear anomalies frequency in the oral mucosa and of nicotine dependence in e-cig users compared with smokers of conventional cigarettes, former smokers, and nonsmokers.

To the best of our knowledge, this is the first study to evaluate MN and metanuclear anomalies in e-cig users compared with former smokers of conventional cigarettes.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee of the Institute of Science and Technology, São Paulo State University (ICT-UNESP) under protocol number 1.033.312/2015 PH/CEP. The inclusion criteria adopted were absence of any history of oral malignancy and absence of any visible clinical signs of alteration at the site to be evaluated. The criteria for noninclusion were consumption of other forms of tobacco (handmade cigarettes, country-style cigarettes, cigars, and pipes), any previous cancer treatment including surgery or radiotherapy and/or chemotherapy in any organ or system, and alteration of the oral mucosa.

Material was obtained from 4 groups of patients as follows:

1. E-cig group: Twenty electronic cigarette users with a history of at least 5 months of use recruited from invitations in social networks
2. Smoker group: Twenty-two smokers of conventional cigarettes attending the Outpatient Smoking Cessation Program of the Heart Institute (Incor), Faculty of Medicine, University of São Paulo (FMUSP), coordinated by Dr. Jaqueline Scholz
3. Former smoker group: Twenty-two patients undergoing smoking treatment with abstinence for at least 1 year and a maximum of 2 years attending the Outpatient Smoking Treatment Program of the Heart Institute (Incor), Faculty of Medicine, FMUSP, coordinated by Dr. Jaqueline Scholz
4. Control group: Twenty-seven consecutive patients who were nonsmokers attending the outpatient clinic of the Department of Biosciences and Diagnostics of the ICT, Unesp, São José dos Campos, São Paulo, coordinated by Dr. Janete Dias Almeida

After being informed about the proposition and conditions of this study, those who accepted participating in the research signed the free and informed consent form in 2 ways.

Data from the participants in the e-cig and control groups were obtained at the time of collection. The data for the smoker and former smoker groups were obtained from the medical records of these patients. Nonetheless, the authors faced challenges associated with missing data in these records.

The evaluation of nicotine dependence was performed by means of the Issa Situational Consumption Score (ISCS).[17] The ISCS consists of 4 questions whose answers generate a score between 0 and 4 that represents the level of dependence: low (up to 1 point), moderate (2-3 points), and high (4 points). The ISCS could be applied to our e-cig, smoker, and former smoker groups, because it quantifies only habits related to dependence; it does not quantify cigarette consumption. In the former smoker group, the data refer to answers given at the beginning of smoking cessation treatment.

The quantification of the smoking load was performed by the calculation of pack-years. Included in the smoking profile analysis were questions regarding current consumption of cigarettes; age of onset; type, quantity, and time of use; smoking load; number of previous cessation attempts; and medical aid for cessation.

The evaluation of cigarette consumption for the e-cig, smoker, and former smoker groups was complemented with the carbon monoxide (CO) concentration of exhaled air, using piCO + Smokerlyzer equipment (Bedfont Scientific Ltd, Maidstone, UK). This equipment measures the CO concentration from a breath of air provided by the participant, classifying it as follows: nonsmokers, 0-6 ppm; borderline, 7-9 ppm; and smokers +10.[18] The e-cig group was measured at the time of collection. In the smoker group, data were obtained from the medical records of smoking patients who had not yet started treatment for smoking cessation. In the former smoker group, data were also obtained from medical records; however, they consist of the evaluation performed on patients after smoking cessation.

The PTS Detect Cotinine System (PTS Diagnostics, Indianapolis, IN, USA) was used to evaluate the capillary blood cotinine concentration of individuals in the

e-cig group for the objective test of nicotine consumption. Cotinine is a biomarker of nicotine ingestion because it represents about 75% of by-products from nicotine, has a longer plasma half-life, and appears in higher concentrations in the blood than nicotine.[19,20] The evaluation was done using blood obtained by capillary puncture (fingertip). Blood was collected and measured by the manufacturer as follows: nonsmokers, <25 ng/mL; possible passive exposure, 25-40 ng/mL; mild tobacco use, 41-199 ng/mL or 1 to 9 cigarettes/d; and heavy tobacco consumption, >200 ng/mL or >10 cigarettes/d.

Exfoliative cytology was performed using a cytobrush for the collection of material from the lateral border of the tongue and mouth floor without previous use of mouthwashes.[21] The smears obtained were fixed with alcohol spray and treated with Feulgen staining as follows. The slides were washed in 95% alcohol and then transferred directly to 5 N hydrochloric acid at room temperature for 5 minutes. The material was transferred and incubated in Schiff's reagent for 90 minutes at 4˚C. Then, 3 consecutive washes were performed in distilled water. After immersion, the material was washed 3 times with absolute alcohol. The slides were clarified with xylol.

The slides were evaluated using an optical microscope, and the cells had a magenta- colored nucleus. The entire slide was visualized at × 400 magnification, and when the presence of MN was detected, confirmation was performed at × 1000 magnification. Approximately 1500 cells were evaluated in each sample. MN analysis consisted of the evaluation of the number of cells with 1 MN, more than 1 MN, total micronucleated cells, and total MN, according to the criteria of Tolbert et al.[15] One cell can contain more than one MN, so we counted the total cells with the presence of MN (regardless of the number of MN in each one) and the total MN present in the same sample (regardless of the number of cells). The metanuclear anomalies (karyolysis, karyorrhexis, binucleation, broken egg, and nuclear bud) were evaluated according to Tolbert et al.,[15] Oliveira et al.,[22] and Dutra et al.[23] (Figure 1).

For the comparison of nonparametric data, exploratory analyses were performed with the Kruskal-Wallis test with Dunn's test as a post hoc method for multiple comparisons. For the correlations and associations between the data, Spearman's correlation test and Fisher's exact test were used, respectively. A significance level of 5% was adopted for all tests. In the case of missing data, the participant was not included in the analysis of the missing variable.

RESULTS

Sample profiling

Information on age, sex, education level, and alcohol consumption are shown in Table I.

Regarding the time of e-cig use, it ranged from 5 to 96 months (8 years), representing an average total use of 31.45 ± 28.44 [18] months (mean ± standard deviation [median]). In the e-cig group, 2 patients reported sporadic use of illicit substances (once or less than once per month), and 2 reported having previously used them. Nicotine concentration of the e-cig group is available in Table II.

In the e-cig group of 20 participants, 18 had been conventional cigarette smokers before e-cig use. The information related to the smoking data of the e-cig group (before the use of e-cigs) and of the smoker and

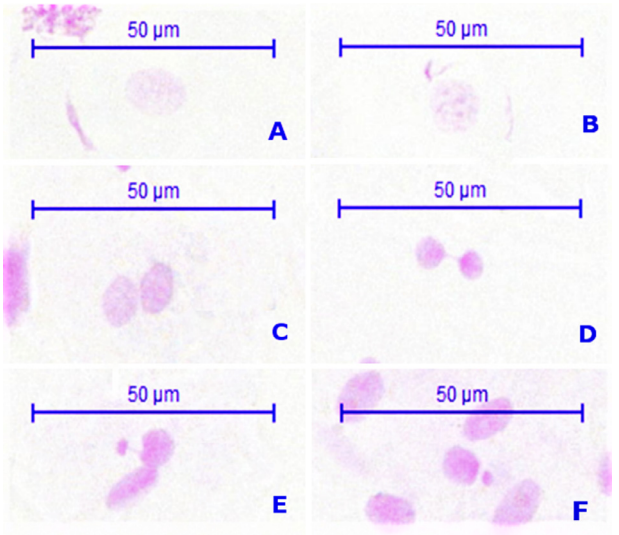

Fig. 1. Oral mucosa cells exhibiting (A) karyolysis; (B) karyorrhexis; (C) binucleation; (D) broken egg; (E) nuclear bud; (F) micronucleus (Feulgen staining).

Table I. Sample profiling with information on sex, age, education level, and alcohol consumption

	E-cig	*Smoker*	*Former smoker*	*Control*
Sex				
Men	14	10	13	13
Women	6	12	9	14
Age				
Mean ± SD	41.5 ± 13.03	51.45 ± 11.27	58.91 ± 10.24	56.48 ± 12.93
Min-max	17-60	29-70	38-73	30-80
Education level				
None	0	1	1	**0**
Primary	1	2	11	4
Secondary	4	8	6	12
Higher education	13	4	4	4
No data	2	7	0	7
Alcohol consumption				
No. of consumers	15	6	9	8
No data	0	6	0	0
Typical number of daily doses of alcohol				
1 to 2 doses	2	3	1	8
3 to 4 doses	5	3	6	0
5 to 6 doses	2	0	2	0
10 or + doses	6	0	0	0

max, maximum; min, minimum; SD, standard deviation.
Alcohol dose: distillate, 36 mL; beer, 350 mL; wine, 120 mL.

former smoker groups (before cessation of smoking), such as number of cigarettes consumed per day, previous smoking time, concentration of CO (ppm) expired, and ISCS, was evaluated using the Kruskal-Wallis test (Table III). In the comparison of the tobacco consumption of the e-cig, smoker, and former smoker groups, there was a statistical difference between the groups regarding time of conventional cigarette use, expired CO, and ISCS. In the smoker group, 8 patients were disregarded in the ISCS analysis due to missing data.

The evaluation of the cotinine concentration using the PTS Detect Cotinine System apparatus is shown in Table II. In 8 cases, it was not possible to quantify the concentration of cotinine in the blood due to sample failure analysis or nonacceptance by the participant. Spearman's correlation test with 5% significance was applied to evaluate the correlation between cotinine concentration and daily frequency in mL/d of e-cigs ($r = 0.388$; $P = 0.213$), as well as the concentration of nicotine added in the e-cig cartridge ($r = 0.203$; $P = .527$). No difference was found in any of the correlations.

Evaluation of MN and metanuclear anomalies

The results of the comparison of MN frequency and metanuclear abnormalities between the groups using the Kruskal-Wallis test ($\alpha = 5\%$) are demonstrated in Figure 2. The representative panorama for the cytopathological outcomes investigated is provided in Figure 3.

Quantitative variables

The frequency of MN and metanuclear abnormalities in the e-cig, smoker, former smoker, and control groups was correlated by Spearman's correlation test ($\alpha = 5\%$), with the following quantitative variables: number of cigarettes per day, time of use of conventional cigarettes and smoking load, concentration of expired CO (Smokerlyzer), and the ISCS. For e-cigs, the following variables were also evaluated: use of e-cigs (in mL/d), concentration of nicotine (in mg)

Table II. Distribution of individuals from the e-cig group regarding use of e-cigs with or without nicotine and quantification by PTS Detect Cotinine System

PTS DETECT COTININE SYSTEM			
PTS Scale	No. of individuals	Nicotine concentration in e-cig	Frequency (mL/d)
<25 ng/mL	3	0 and 18 mg	4 and 5 mL/d
25-40 ng/mL	1	6 mg	?
41-99 ng/mL	1	1.2 mg	12 mL/d
<200 ng/mL	7	2 and 4 mg	5, 10, and 30 mL/d

The PTS Scale is in accordance with the manufacturer's instructions. Nicotine concentration in e-cigs refers to the concentration of nicotine contained in the e-cig cartridge. Frequency (mL/d) corresponds to how much the individuals consume in mL of the liquids contained in the cartridge per day. "?" represents an individual who could not quantify the daily frequency in mL/d.

Table III. Mean tobacco consumption and dependence-level data for e-cig, smoker, and former smoker groups

	E-cig	*Smoker*	*Former smoker*	*P value*
No. of cigarettes/d	23.26 ± 15.53 [20]	17.05 ± 9.844 [15]	18.55 ± 11.38 [16.5]	0.2239
Time of use (y)	21.58 ± 14.17 [20]	33.09 ± 11.86 [33.5]	39.86 ± 13.22 [40]	0.0012
Smoking load	29.49 ± 26.05 [27]	29.76 ± 22.09 [23.75]	37.45 ± 27.71 [28,50]	0.5069
CO (ppm)	4.58 ± 3.59 [4]	10.14 ± 8.72 [8.5]	1.727 ± 0.77 [2]	<.0001
ISCS	2.05 ± 1.18 [2]	2.86 ± 0.95 [3]	2.909 ± 0.87 [3]	0.0296

Kruskal-Wallis test ($\alpha = 5\%$) for number of cigarettes per day, time of use, smoking load, carbon monoxide (CO), and Issa Situational Consumption Score (ISCS). Data are presented as mean ± standard deviation [median]. * (P <0.05).

contained in the e-cig cartridge, and cotinine concentration (in ng/mL) measured by the PTS Detect Cotinine Test System (PTS Diagnostics, Indianapolis, IN, USA). The results showed significant correlations in the e-cig group between nuclear bud and time of use of conventional cigarettes ($r = 0.609$; $P = 0.006$), previous smoking load ($r = 0.499$; $P = 0.029$), and concentration of cotinine (in ng/mL) ($r = 0.615$; $P = 0.033$) and between CO expired and karyorrhexis ($r = 0.465$, $P = 0.045$) and binucleation ($r = 0.593$, $P = 0.007$). In the former smoker group, the significant correlations were between karyolysis and number of cigarettes/d ($r = 0.530$; $P = 0.011$), previous smoking load ($r = 0.632$; $P = 0.002$), and ISCS ($r = -0.438$; $P = 0.042$). There were no significant correlations in the smoker group.

One individual in the e-cig group was excluded from these correlations analyses because of lack of necessary information regarding the previous smoking burden. For the statistical analysis of cotinine concentration in the blood, 12 cases were considered in total. As mentioned above, there were 8 cases in which it was not possible to measure capillary cotinine concentration due to either sample failure or the participant's refusal to take the test. In the 12 cases where measurement was possible, 24 ng/mL was used when the PTS Detect Cotinine System was <25 ng/mL and 201 ng/mL when the dosage was >200 ng/mL; the other concentrations (between 25 and 200 ng/mL) were used with the exact number displayed by the apparatus. In the analysis of the frequency of use of e-cigs, 3 individuals were not able to quantify the use in mL/d; therefore, the minimum dose was considered among the participants of the group of 3 mL/d for these participants.

Qualitative variables

Analysis of the association between the frequency of MN and metanuclear abnormalities and the variables related to sex and intraoral location of sample collection was also performed using Fisher's exact test ($\alpha = 5\%$) for all groups. The results obtained from this analysis showed that there was a significant association in the e-cig ($P = 0.0098$) and smoker ($P = 0.026$) groups between the sample collection site and nuclear bud, with a greater association of the tongue.

DISCUSSION

The increased frequency of MN, binucleation, broken egg, and nuclear bud are considered indicators of genotoxicity,[10,15] whereas karyolysis and karyorrhexis are indicators of cytotoxicity.[10,12,15,16] Thus, in the present study, the e-cig group showed genotoxicity and cytotoxicity. It presented a significantly higher number of broken eggs than the smoker group and more karyolysis, binucleation, broken eggs, and nuclear buds than the former smoker and control groups. The former smoker group showed no significant cytotoxicity, as there were no statistically significant differences in the frequency of MN and metanuclear anomalies compared with the control group.

Regarding the frequency of MN, no significant difference was observed between the e-cig group and the other groups, which differs from the results of Franco et al.,[24] in which the frequency of MN in the e-cig group was significantly smaller than in the smoker group. Nonetheless, it is important to consider that the participants in the study by Franco et al.[24] did not consume alcohol. Oliveira et al.[22] found an increase in the frequency of MN in smokers who used alcohol abusively, indicating that alcohol can cause cellular genotoxicity. In addition, alcohol favors the penetration of carcinogenic substances into the oral mucosa, acting as a promoter in carcinogenesis,[22] and contains substances such as acetaldehyde, which can induce oxidative stress.[25] This suggests that the genotoxic effects found in the e-cig group in the present study may have been enhanced by alcohol consumption.

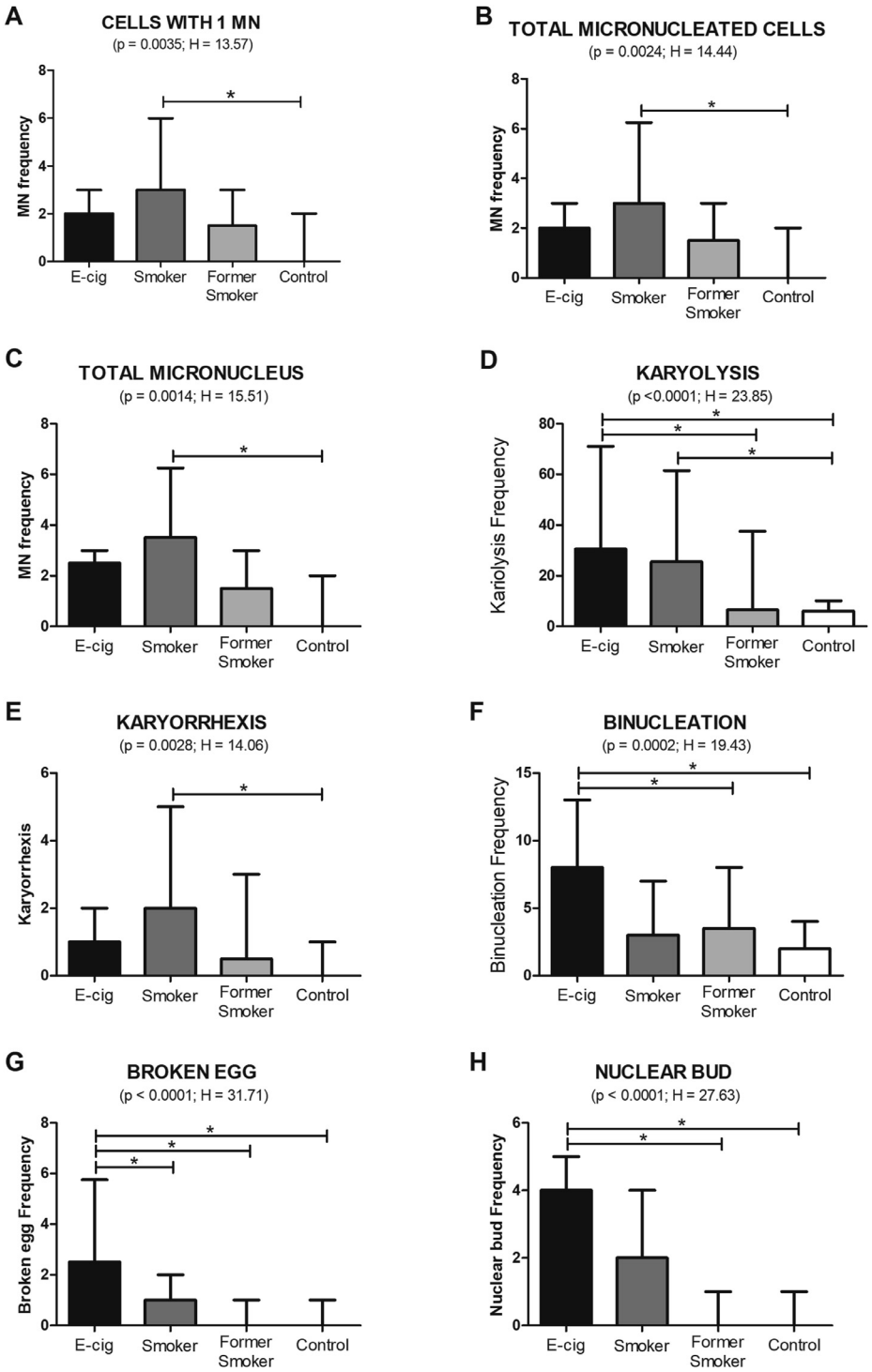

Fig. 2. Graphs showing the comparison between MN frequency and metanuclear abnormalities in the e-cig, smoker, former smoker, and control groups, using the Kruskal-Wallis test with Dunn's test as a post hoc method for multiple comparisons. H represents the coefficient of the Kruskal-Wallis test ($P < .05$); Dunn's test is represented with an asterisk. (A) Cells with only one Micronucleus - the smoking group was different from the control group; (B) Total Micronucleated cells - the smoking group was different from the control group; (C) Total Micronucleus - the smoking group was different from the control group; (D) Karyolisis - the e-cig group was different from the former smoker and control group, and the smoker group was different from the control group; (E) Karyorrhexis - the smoker group was different from the control group; (F) Binucletion - the e-cig group was different from the former smoker and control group; (G) Broken Egg - the e-cig group was different from all groups; (H) Nuclear Bud - The e-cig group was different from the former smoker and control group.

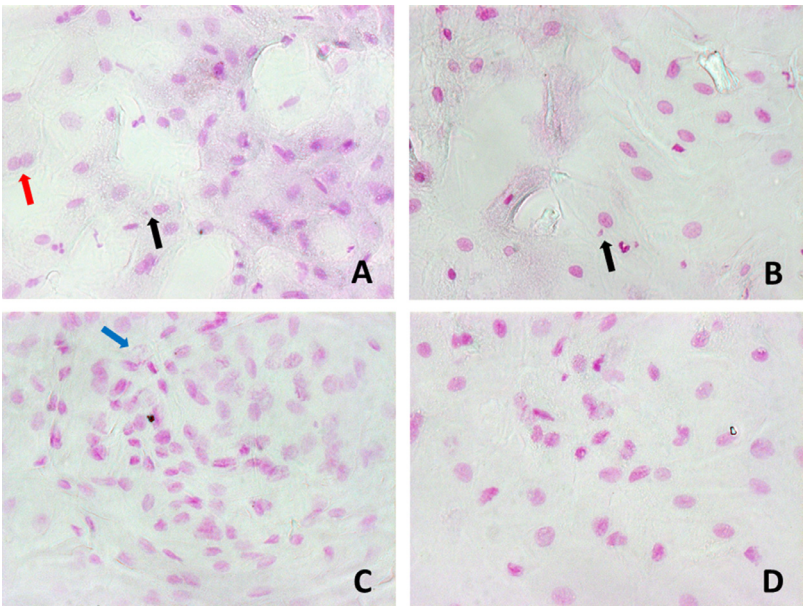

Fig. 3. Representative group areas for comparative panorama of the cytopathological outcomes investigated. (A) E-cig group MN (black arrow) and binucleation (red arrow). (B) Smoker group MN (black arrow). (C) Former smoker group karyolysis (blue arrow). (D) Control group. Feulgen staining; original magnification, × 400.

Although it was not possible to recruit participants in the e-cig group who had never smoked and had consumed no alcohol, the profile of the evaluated group is interesting, as it reflects the reality commonly found, considering that e-cigs are used by some people to quit smoking.[26] The fact that the e-cig group obtained a higher frequency of metanuclear anomalies than the former smoker group draws attention and suggests that the use of e-cigs makes it difficult to reduce the damage caused by previous smoking and perhaps can boost such damage. In addition, another factor that may have contributed to this difference is the harmful substances, such as formaldehyde, acetaldehyde, and acrolein, that may be by-products of the chemical reactions that occur with the increase in the temperature in liquids in these devices.[1,3,5,27]

In relation to what was observed in the smoker and former smoker groups, it is evident that the consumption of tobacco promotes genotoxic and cytotoxic damage in the cells of the oral mucosa, as already established in the literature.[28-30] Notably, the decrease of the harmful effects of tobacco smoking with smoking cessation treatment has also become clear.

In the study by Nersesyan et al.,[28] one of the groups of smokers presented a number of MN 3 times greater than the control, whereas in the analysis of binucleation and broken eggs, it was 7 and 17 times larger, respectively. In the present study, this sensitivity was noticed in the e-cig group, since there was no significant difference in the MN frequency comparison, but there was a difference for the metanuclear anomalies.

The majority of participants recruited in the e-cig group reported that they had quit smoking conventional cigarettes with the help of e-cigs. Truman et al.[26] also found that the use of e-cigs favored smoking cessation in the majority of study participants.

Regarding nicotine dependence, the present study identified a high concentration of cotinine in the blood of individuals in the e-cig group, equivalent to the nicotine consumption of more than 10 conventional cigarettes per day. The level of nicotine dependence in the e-cig group was significantly lower than in the former smokers (beginning of treatment for smoking cessation, still smokers), as observed in the study by Foulds et al.[9] Although nicotine causes harmful effects and promotes addiction, this substance appears to play a minor role in the development of smoking-related diseases compared with the other toxic substances contained in conventional cigarettes.[4,19,31,32]

The presence of nuclear buds directly related to the concentration of cotinine in the blood of e-cig users may be indicative that nicotine promotes genotoxicity. In contrast, the correlation between ISCS and karyolysis was reversed in the former smoker group. It is important to consider that the data from the ISCS refers to the beginning of smoking cessation treatment that was evaluated at least 1 year before the collection for MN evaluation and metanuclear anomalies, which may be the reason for this reversal. In contrast, Nersesyan et al.[28] evaluated exfoliated oral cells and suggested that nicotine may provide protection against nicotine-depleted cells from cigarette DNA because they found an inverse correlation between nicotine levels in

cigarettes and MN frequency, as well as some types of metanuclear abnormalities (karyolysis, karyorrhexis, and binucleation).

The directly proportional correlation between previous tobacco consumption and metanuclear anomalies observed in the e-cig group and the former smoker group can be attributed to several carcinogenic substances released by cigarette smoking to which these individuals were previously exposed. Such correlations suggest, therefore, that the previous consumption of conventional cigarettes still generates an influence on the DNA of the cells of the oral mucosa. Nersesyan et al.[28] found no correlation between toxicity and cumulative exposure indicators, but they found that karyolysis, karyorrhexis, and binucleation increased according to the greater exposure to tar present in cigarettes.

Smoking is considered the most likely cause for increased expired CO; however, this increase can occur due to other factors, such as exposure to secondhand smoke, exposure to air pollution, consumption of alcohol, and smoking of other forms of tobacco and cannabis (marijuana).[33] In the present study, as expected, the expired CO concentration in the e-cig group was significantly lower than in the smoker group, considering that e-cig devices do not burn tobacco.[1] Former smokers, no longer exposed to burning tobacco, had a lower concentration of expired CO, as observed in the studies by Stelmach et al.[34] and Goldstein et al.[35] Similar levels were expected for the e-cig and former smoker groups, given that both had experienced smoking cessation for some time. However, expired CO was statistically higher in the e-cig group than in the former smoker group. The authors suppose that this difference may have occurred due to passive smoking by some when consuming e-cigs in the same areas used by conventional smokers, alcohol consumption, and unreported information regarding sporadic use of conventional or cannabis cigarettes.

Furthermore, it can be presumed that the increase of CO had a genotoxic effect on the cells of the oral mucosa in the e-cig group, as a directly proportional correlation was seen of the CO concentration expired with karyorrhexis and binucleation. This is due to the fact that apoptosis-induced karyorrhexis and binucleation are related to the initiation of carcinogenesis by genotoxic agents.[15] Moreover, there may be a relationship to the fact that expired CO promotes a reduction of oxygen transport in the blood,[35] and decreased oxygen supply in tissues may favor cell instability leading to cell death.

The fact that the localization of the tongue is more associated with the presence of nuclear bud in the e-cig group and in the smoker group can be justified by the fact that the tongue, at the time of vaporization, establishes more contact with the vapor and heat than the floor of the mouth. In addition, nuclear buds indicate genotoxic changes that may be indicative of carcinogenesis,[15] and the tongue is the site of the worst prognosis when it is affected by carcinoma.[36]

The limitations of the present study are related to the challenges of missing data in medical records and to the difficulty in recruiting participants for the e-cig group who do not use concomitant alcohol, besides the question related to standardizing the concentrations of liquids used and the necessity of a scale for nicotine dependence. However, this study contributes to the discussion of new aspects related to e-cigs, namely nicotine dependence, the comparison of cytogenetic and cytotoxic damage in the cells of the oral mucosa in e-cig users and former smokers, and the influence of alcohol associated with the use of e-cigs. It is important to emphasize that the present study evaluated the safety of e-cigs only in the mucosa cells of the tongue and the floor of the mouth.

In summary, e-cig users presented genotoxicity and cytotoxicity in the oral mucosa cells. The damage cannot be attributed solely to the use of the e-cigs, since most vaporizers also consumed alcohol and had a history of using conventional cigarettes. However, smoking cessation without the use of e-cigs has been shown to be safer and more effective in reducing damage. Further studies are needed to assess the long-term effects of e-cigs.

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PRESENTATION

This study has been presented previously at the 2nd International Conference on Dentistry and Oral Health, April 2019, Milan, Italy, and III ODONTOMEETING/IX CEAJO, October 2019, São José dos Campos, Brazil.

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