Evaluation of micronuclei, cytomorphometric and cytologic changes of the oral mucosa in hookah and cigarette smokers

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Objective. To analyze the effect of hookah and cigarettes on the oral mucosa of smokers through the use of exfoliative cytology.
Study Design. Smear samples were collected by exfoliative cytology from the tongue of 33 hookah smokers, 22 cigarette smokers, and 30 non-smokers. The selected analyses include micronuclei (MN), metanuclear anomalies, epithelial maturation, and cytomorphology (nuclear area [NA], cytoplasmic area [CA], and NA/CA ratio).
Results. The largest differences observed for MN and metanuclear anomalies were between cigarette smokers and the control group (notably 1 MN $P = .04$; total cells with MN $P = .039$; total MN $P = .042$; karyorrhexis and binucleation, $P = .0001$). The hookah group, compared with the control group, showed the greatest differences for karyolysis ($P = .0023$), binucleation ($P = .0003$), and broken egg ($P = .008$). Significant differences were found between the smokers and the control groups regarding changes in the superficial cell without nucleus, perinuclear halo, vacuolization, color change, mucus, and keratohyalin granules. There was a significant increase in the NA and NA/CA ratio in the smoker groups.
Conclusion. This study showed that a combined analysis of exfoliative cytology associated with other diagnostic methods is a useful tool for studying oral carcinogenesis. Hookah and cigarettes showed similar effects in terms of displaying substantial cytogenetic and cytotoxic damage. (Oral Surg Oral Med Oral Pathol Oral Radiol 2024;137:640−650)

Smoking in its various forms of consumption is one of the greatest threats to public health worldwide, giving rise to the death of more than 8 million people per year.[1] Despite this, 1.3 billion people continue to consume tobacco products, and it is estimated that annually, about 11% of men and 6% of women worldwide lose their lives due to tobacco consumption.[1] Most people are unaware of the consequences of tobacco consumption and the risk it represents of the onset of heart and respiratory diseases and more than 20 types of cancer, including oral cancer. The latter represents approximately 10% of all worldwide cancer cases.[2]

Even though cigarettes are still the predominant form of tobacco consumption worldwide,[3] there has been an exponential increase in the consumption of hookah not only in Eastern countries and India but also in the West, and this increase has been more prevalent in the younger population who mistakenly believe that hookah is less harmful and addictive than cigarettes.[4,5] Data from 73 countries showed that the prevalence of use of hookah among 335,062 adolescents aged 12 to 16 years was 6.9%, and it was most prevalent in Eastern Mediterranean and European countries.[6] In 2022, more than 3 million youngsters reported using a tobacco product, and almost 150,000 reported that they had smoked hookah in the previous 30 days.[7]

Both hookah and cigarettes contain nicotine in their composition, producing addiction and detrimental

Statement of Clinical Relevance

The exfoliative cytology, in association with the micronuclei test, cytomorphometry, and Papanicolaou analysis, is an important tool for the early identification of oral carcinogenesis in tobacco consumption. Furthermore, the cellular changes observed in the oral mucosa of hookah users were similar to those of cigarette smokers. These similarities can be used to bring awareness among young people about the negative effects of hookah.

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effects in the oral cavity through the action of carcinogenic agents and toxic substances such as nitrosamines, polycyclic aromatic hydrocarbons, volatile aldehydes, benzene, nitric oxide, heavy metals, etc.[8] In addition, hookah releases a greater amount of carbon monoxide and promotes greater exposure to smoke than cigarettes.[9]

At present, oral cancer incidence worldwide involves 377,713 new cases and 177,757 deaths annually,[10] and squamous cell carcinoma (SCC) is the most prevalent malignant neoplasm in the oral cavity. Smoking tobacco poses the highest risk factor associated with its development.[11] The SCC most often affects the region of the tongue and floor of the mouth,[3] men aged over 50 years,[12] and tobacco and alcohol users.[13] These products are considered genotoxic agents that act in the oral mucosa cells, causing damage that may be related to the various stages of carcinogenesis.[14]

Unfortunately, diagnosis of oral cancer has often been delayed because the incipient lesions tend to be small and asymptomatic, making early detection difficult.[15] The treatment of oral SCC lesions at an early stage of development is more effective, highlighting the importance of early diagnosis, in addition to the identification and monitoring of lesions with potential for malignant transformation.[16]

Exfoliative cytology has been frequently used in the early diagnosis of oral cancer because it allows the analysis of exfoliated cells from different layers of the epithelium through light microscopy,[17] highlighting morphologic variations, maturity, and cellular metabolic activity degree.[18]

The micronucleus (MN) test, whose purpose is to identify the frequency of micronucleus and metanuclear anomalies in oral mucosa cells, has been frequently used as a genotoxicity biomarker to evaluate chromosomal tissue damage in tobacco users. MN is a simple, fast, and sensitive method to detect toxicity due to environmental agents, and it is currently being considered for use as a biologic marker. Some studies show that the genotoxic effects of tobacco on the epithelial cells of the oral mucosa are reflected in the increase of MN number in smokers.[19,20] Individuals who use tobacco present genotoxic and cytotoxic damages that interfere with the mitosis process, which in turn leads to MN formation.[21]

The cytomorphometry is another technique frequently employed associated with exfoliative cytology to analyze the effects of tobacco on epithelial cells, presenting alterations in quantitative parameters such as nuclear (NA) and cytoplasmic areas (CA) and in the nucleus/cytoplasm relationship (NA/CA). The latter is considered an important cell morphology alteration present in the process of carcinogenesis.[22]

Based on the above, it is relevant to analyze the effect of tobacco in the oral mucosa of smokers with different consumption habits, such as hookah and manufactured cigarette smokers, through genotoxic effect, morphologic variations, cell maturation, and cytomorphometric changes of these carcinogens in oral epithelial cells. It is also important to analyze the correlation between the changes found in the clinical data and the smoking profile of the patients.

MATERIALS AND METHODS

This study was conducted in accordance with the declaration of Helsinki and approved by the Research Ethics Committee of the University of Mogi das Cruzes, under approval number 2,627,150. The inclusion criteria adopted were adult individuals over 18 years of age, of both sexes, without a personal history of oral malignant neoplasia nor visible clinical signs of any alteration in the evaluated site, and free from systemic diseases. The criteria for non-inclusion were samples from patients who have already undergone any type of surgical, radiotherapy, and/or chemotherapy oncological treatment in any organ or system. The exclusion criteria included cases of degraded or insufficient samples or, cases with incomplete forms that hinder the correct identification and interpretation of data.

The samples were collected through exfoliative cytology in 3 groups: (1) the hookah group, composed of 33 individuals with a hookah smoking habit, exclusively, at least twice a week for 2 years; (2) the cigarette group composed of 22 individuals with a cigarette smoking habit, exclusively, at least 10 cigarettes per day[23] for 2 years; and (3) the control group formed of 30 individuals with non-smoking habit, and who have not been regular passive smokers throughout their entire lives.

All patients received detailed information about the objective and procedures of the study and signed the free informed consent form. They were evaluated clinically and answered a questionnaire about general data and their general health condition. For the analysis of the smoking profile, questions were asked about tobacco consumption at the time of the test, age at onset, type, quantity, time of use, and consumption of other substances. Patients were also submitted to the AUDIT test (Alcohol Use Disorder Identification Test), an instrument used to assess alcohol consumption and its related risks.[24]

The exfoliate cytology specimens were collected 10 minutes after a rinse with distilled water 60 minutes after food intake and at least 12 hours after alcohol intake. The smear was collected with a cytobrush (Adlin) from the right lateral border of the tongue, applied on glass slides, and immediately fixed with an alcoholic spray Kolplast, São Paulo, Brazil.[25]

For MN and metanuclear anomalies analysis, 2 slides were stained by the Feulgen method without the FastGreen counterstain (Sigma). After this, slides were examined under light microscopy initially at 400 × magnification and then at 1000 × magnification for the confirmation of MN presence. Approximately 3,000 cells were evaluated in each sample. The count included only cells with intact nuclei and with smooth and distinct nuclear perimeters, as recommended by Tolbert et al.,[26] including analyzing at least 1000 cells on a slide to obtain more accuracy. For the MN count, the applied criterion was the presence of a homogeneous surrounding halo, less than a 1/3 of the diameter of the associated nucleus, the intensity of Feulgen staining similar to the nucleus, and the same focal plane on microscopy and without presenting any connection with the nucleus. Additionally, the evaluation of the presence of metanuclear anomalies was assessed: karyorrhexis, karyolysis, binucleate cells, broken-eggs, and nuclear bud.[27] This phenomenon is represented by a nucleus when divided into 2 portions in close proximity, typically one larger than the other, connected by a thin layer that is reported to be Feulgen negative.[28]

For the Papanicolaou evaluation,[29] 2 slides were stained using the Papanicolaou stain method and analyzed in all their extension, semi-quantitatively in the smear by light microscopy at 200 × and 400 × magnification. The Papanicolaou method allows the evaluation of morphologic characteristics and the pattern of cellular epithelial maturation. In the qualitative criteria, the histologic findings are rated as class 0 (not representative/inadequate analysis), class I (normal, absence of abnormal or atypical cells), class II (normal with inflammatory alterations), class III (atypical, suggestive or but inconclusive of malignancy), class IV (strongly suggestive, but not conclusive for malignancy), and class V (cytology conclusive for malignancy).[29] These were stratified by the Papanicolaou classification, observing the presence of superficial cells with a nucleus; superficial cells without a nucleus; intermediate cells; basal cells; inflammatory infiltrate; inflammatory alterations (perinuclear halo, vacuolization, and color change); red blood cells, mucus, bacterial colonies, Candida hyphae, keratohyalin granules, and cellular atypia. Occurrence scores were applied as follows: 0 = absent; 1 = up to 25%; 2 = 25%-50%; 3 = more than 50%.[30]

For cytomorphometric analysis, 2 slides were stained using the Papanicolaou method, and 100 cells with well-defined cytoplasmic and nuclear limits were randomly selected and photographed at 400 × magnification using light microscopy. The CA and NA were measured in μm^2 using the Image J software (National Institutes of Health), and the NA/CA ratio of the selected cells was calculated.

To minimize bias resulting from knowledge of the groups exposed to tobacco, the observer who selected and measured the cells on slides did not have knowledge of which group cells belonged to, characterizing the study as single blind.

Data were subjected to statistical analysis using the PRISM software (GraphPad, Inc., version 5.03, 2010). Descriptive statistics involved mean and SD calculation. Inferential statistics involved the Kruskal−Wallis test, Dunn's multiple comparison test, and association among variables performed employing the Spearman correlation test. In all analyses, the chosen significance level was 5%.

RESULTS

The results from the study of the effect of tobacco on the oral mucosa of hookah and cigarette smokers, as well as the control group, are presented below. They include analysis of genotoxic effect, morphologic variations, cell maturation, and cytomorphometric changes in the oral epithelium.

As described, exfoliative cytology samples were collected from 85 individuals, where 33 were hookah smokers, 22 were cigarette smokers, and 30 were nonsmokers (control group). Information on age, sex, tobacco consumption, time of consumption, and alcohol-related risk score of studied patients in the 3 groups are shown in Table I.

Figure 1 shows the most common cytologic findings observed in stained cells by the Feulgen method. Table II shows the *P* value in relation to these variables examined using the Kruskal−Wallis test and Dunn's multiple comparison test. In Supplemental Figure S1, the distribution of cases in relation to the frequency of MN and metanuclear anomalies within the studied groups is observed.

The higher frequency of MN was found only in the cigarette group compared with the control, and there were no significant differences in the frequency of more than 1 MN, total cells with MN, and total of MN between the smokers' groups and the control group. In addition, the MN mean was low, between 0 and 1, for most individuals in all 3 studied groups. The largest differences observed for MN were between cigarette smokers and the control group (notably 1 MN $P = .04$; total cells with MN $P = .039$; total MN $P = .042$), and for metanuclear anomalies (karyorrhexis and binucleation $P = .0001$). The hookah group, compared with the control group, showed the greatest differences for karyolysis ($P = .0023$), binucleation ($P = .0003$), and broken egg ($P = .008$).

Regarding the correlation between the frequency of MN and metanuclear anomalies and clinical data, the Spearman correlation test ($\alpha = 5\%$) in the hookah group presented a significant correlation between the time of

Table I. Profiles for age, sex, tobacco consumption, duration of tobacco consumption, and alcohol-related risk score of patients in the 3 studied groups

		Hookah group n = 33	*Cigarette group n = 22*	*Control group n = 30*
Age (y)	Average	21.18	29	25.6
	SD	2.7	5.56	7.16
	Range	18-31	20-38	18-43
Sex	Male	9	9	7
	Female	24	13	23
Tobacco consumption	Average*	5.68	14.59	NA
	SD*	5.27	4.99	NA
Tobacco consumption period (y)	Average*	4	12.00	NA
	SD*	1.73	4.74	NA
Alcohol-related risk score	Low-risk drinking	20	16	28
	At-risk drinking	9	5	2
	Hazardous drinking	2	1	0
	Probable dependence	2	0	0

*Tobacco consumption for the Hookah Group = number of sessions per week and for the Cigarette Group = number of cigarettes per day.*NA*, not applicable.

hookah consumption and binucleation (Spearman's correlation coefficient = −0.455, *P* value = .008). The cigarette group presented a significant correlation between the AUDIT index and 1 MN occurrence (Spearman's correlation coefficient = −0.525, *P* value = .018), the total number of micro-nucleated cells (Correlation coefficient of Spearman = −0.504, *P* value = .024), the total of MN (Spearman's correlation coefficient = −0.485, *P* value = .031), and karyorrhexis (Spearman's correlation coefficient = −0.533, *P* value = .016), in addition to consumption and karyolysis time (Spearman's correlation coefficient = 0.549, *P* value = .013), use of alcohol and karyorrhexis (Spearman's correlation coefficient = 0.489, *P* value = .029), and daily number of cigarettes and nuclear bud (Spearman's correlation coefficient = −0.470, *P* value = .037). The control group did not present a statistically significant correlation.

Figure 2 shows the most commonly observed cytologic findings on slides stained by the Papanicolaou method and according to the Papanicolaou

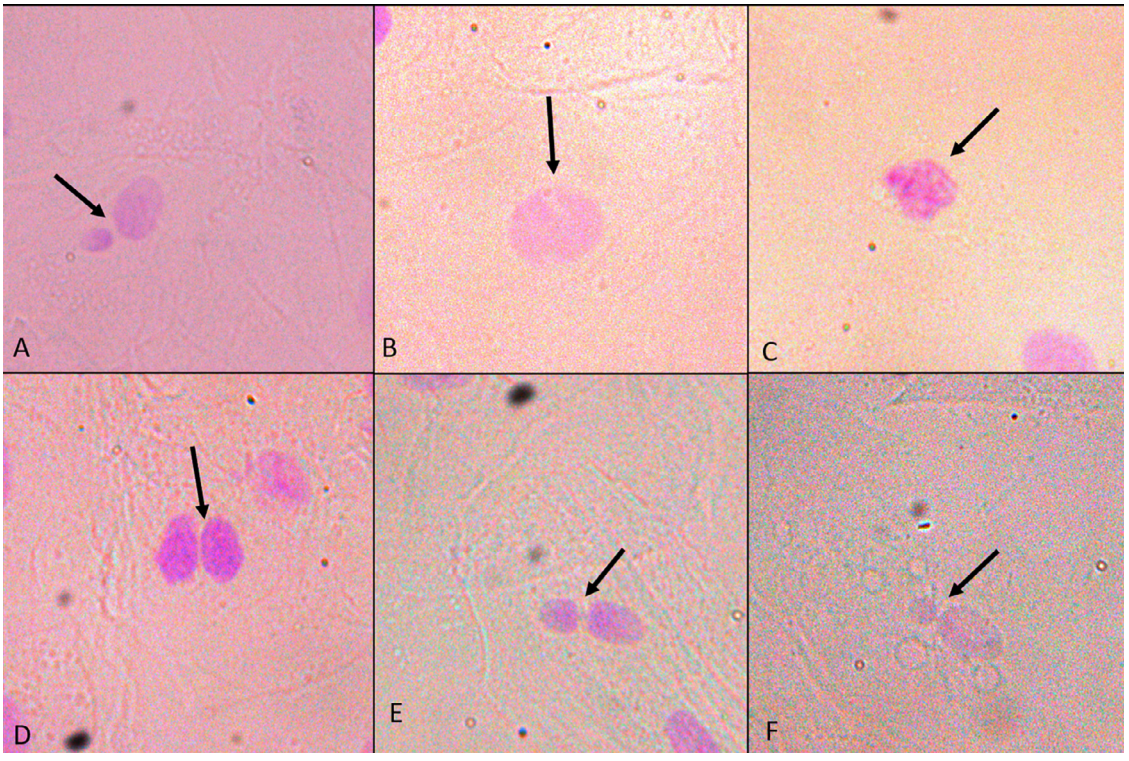

Fig. 1. Oral mucosa cells showing micronuclei and metanuclear anomalies in Feulgen stain. **(A)** Micronucleus. **(B)** Karyolysis. **(C)** Karyorrhexis. **(D)** Binucleation. **(E)** Broken-egg. **(F)** Nuclear bud.

Table II. *P* value distribution in relation to the variables explored for Kruskal−Wallis and the multiple comparison tests against the frequency of micronucleus and metanuclear anomalies

Variables	*Kruskal-Wallis's test*	*Dunn's test*		
		Hookah group × Control group	*Cigarette group × Control group*	*Hookah group × Cigarette group*
1 Micronucleus	.1130	.1728	.0416	.4076
More than 1 micronucleus	.2389	1.0000	.1354	.1279
Total cells with micronucleus	.1083	.1726	.0393	.3945
Total of micronucleus	.0936	.1016	.0417	.5644
Kariolysis	< .0001	.0023	< .0001	.0005
Karyorrhexis	.1749	.3913	.0619	.2634
Binucleation	< .0001	.0003	< .0001	.3120
Broken egg	.0035	.0008	.0821	.1923
Nuclear bud	.2082	.0917	.7531	.2207

Variables with a statistically significant correlation, *P* value ≤ .05.

classification system. In the hookah group, 9.09% of the smears were classified as class 0, 57.57% as class I, and 33.33% as class II. In the cigarette group, 13.63% were classified as class 0, 54.54% as class I, and 31.81% as class II. In addition, in the control group, 3.33% were classified as class 0 and 96.66% as class I. On the other hand, cases in smoker groups that were considered class II, inflammatory changes such as perinuclear halo, vacuolization, and color change were observed, with a statistically significant difference in relation to the control group. Significant differences were also found between the group of smokers and the control group regarding changes in the superficial cell without nucleus, mucus, and keratohyalin granules.

Table III shows the *P* value in relation to the examined variables using the Kruskal−Wallis test and Dunn's multiple comparison test. In variables of superficial cells with a nucleus, basal cell, and candida hyphae, the variance was zero, and the mean was the same in the 3 groups. Supplemental Figure S2 shows the distribution of cases in relation to the changes observed by the Papanicolaou system of classification within the studied groups.

The Papanicolaou analysis showed some association with clinical data: the hookah group presented a positive association only between the consumption time and the presence of perinuclear halo (Spearman's correlation coefficient = 0.410, *P* value = .024). The cigarette group presented a significant correlation between cigarette consumption per day and the presence of intermediate cells (Spearman's correlation coefficient = −0.587, *P* value = .001), between age and atypia's presence (Spearman's correlation coefficient = −0.545, *P* value = .021) and between consumption time and atypia's presence (Spearman's correlation coefficient = −0.477, *P* value = .046). The control group presented a significant correlation between age and the presence of intermediate cells (Spearman's correlation coefficient = 0.493, *P* value = .007).

Table IV shows the mean and SD of NA, CA, and NA/CA ratio in the studied groups. Table V shows the *P* value distribution in relation to the examined variables using the Kruskal−Wallis test and Dunn's multiple comparison tests for cytomorphometric evaluation. Supplemental Figure S3 shows the distribution of cases in relation to the core area (μm^2), cytoplasm area (μm^2), and nucleus/cytoplasm ratio values in the 3 studied groups.

Regarding the correlation between cytomorphometry and clinical data, the cigarette group presented a statistically significant correlation between the AUDIT index and AN (Spearman's correlation coefficient = 0.529, *P* value = .015), between consumption time and CA (Spearman's correlation coefficient = −0.446, *P* value = .044), and between consumption time and ratio NA/CA Spearman's correlation coefficient = 0.549, *P* value = .011). The control group did not present a statistically significant correlation.

DISCUSSION

The development of the present study sought to contribute to the understanding of hookah and cigarette smoking and its effects on oral epithelial cells. It is noteworthy that, to date, there are no publications in the literature that have used 3 different methods of assessing the carcinogenic potential of hookah in oral epithelial cells, such as those adopted in this study, which allows for a better understanding of epithelial tissue changes.

The analysis of MN and metanuclear anomalies is one of the methods performed through exfoliative cytology to investigate the genotoxicity and

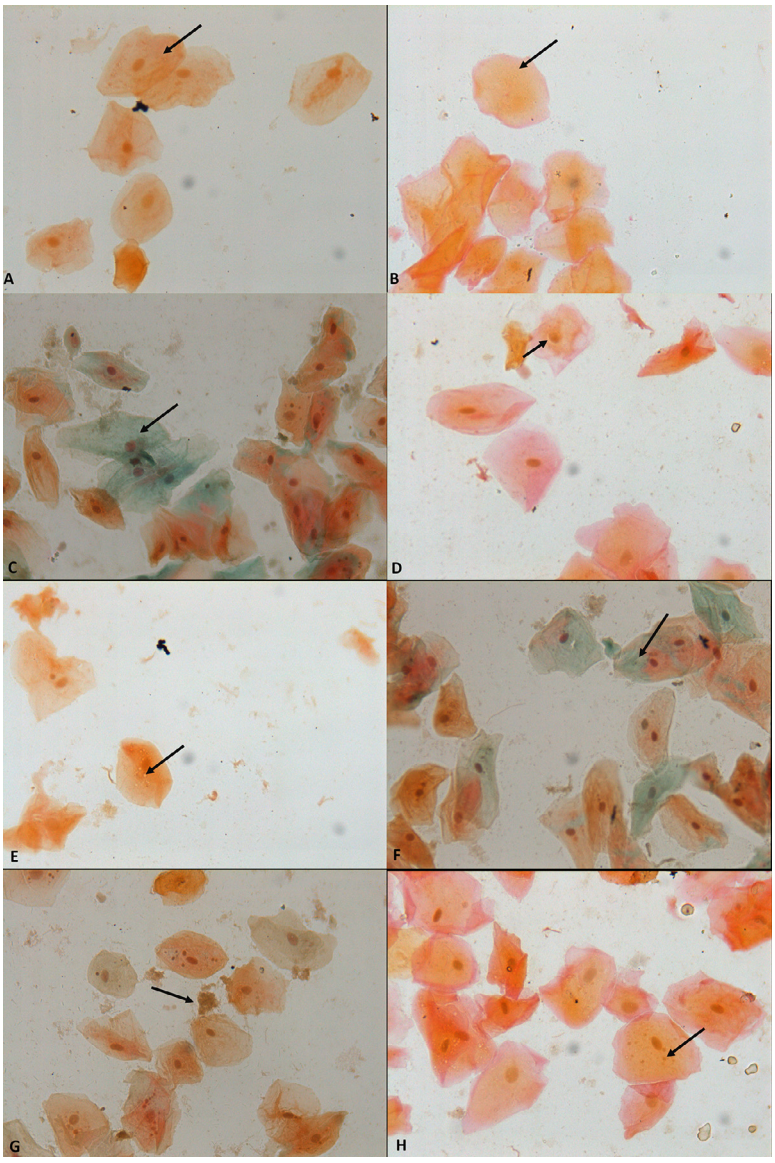

Fig. 2. Most commonly observed cytological findings in cells stained by the Papanicolaou method (400 ×). **(A)** Superficial cell with a nucleus. **(B)** Superficial cells without a nucleus. **(C)** Intermediate cell. **(D)** Perinuclear halo. **(E)** Vacuolization. **(F)** Color change. **(G)** Bacterial colonies. **(H)** Keratohyalin granules.

cytotoxicity of different environmental factors on oral cells,[31] and thus providing a biomarker of damage and indicator of carcinogenic potential in the long term, helping in the diagnosis of cancerous lesions that are already clinically visible and also for the detection of the disease on its early stages.[32] Therefore, there is a higher frequency of MN in smokers due to the genotoxic damage caused by tobacco components that interfere with the mitosis process during the anaphase stage and lead to MN formation.[22,33,34,35]

A significantly greater frequency of MN was found only in the cigarette group in this study, also observed by Pereira da Silva et al.[35] Contrary to our study, the mean number of MN in hookah smokers was significantly higher than the control group in the findings of Rajabi-Moghaddam et al.,[36] Kamath et al.,[19] Derici Eker et al.,[20] and Dehghannezhad et al.,[37] who stated that the effect of hookah genotoxicity is dose-dependent. This can be explained by consumption, which tends to be weekly and not daily, as observed with cigarettes. Cerqueira et al.[38] highlighted that epithelial turnover occurs between 7 and 16 days, and the MN is noticed only 3 weeks after exposure to the genotoxic agent.

A high amount of MN was observed by El-Setouhy et al.[39] and Motgi et al.[40] in the hookah group, whereas in the present study, the mean was between 0 and 1 in most individuals in the 3 studied groups. The

Table III. *P* value distribution in relation to the variables for Kruskal−Wallis and Dunn's multiple comparison tests in findings examined by means of the Papanicolaou method

Variables	*Kruskal-Wallis's test*	*Dunn's test*		
		Hookah group × Control group	*Cigarette group × Control group*	*Hookah group × Cigarette group*
Superficial cell without nucleus	.0408	.0222	.0465	.9777
Intermediate cell	.3576	.1515	.5200	.5312
Perinuclear halo	< .0001	< .0001	< .0001	.4703
Vacuolization	< .0001	< .0001	< .0001	.5881
Color change	< .0001	< .0001	< .0001	.4471
Red blood cells	.5161	.3414	.3151	.8678
Mucus	< .0001	.0003	< .0001	.4615
Bacterial colonies	.0448	.5561	.0574	.0149
Keratohyalin granules	< .0001	< .0001	< .0001	.1250
Cellular atypias	.6439	.7642	.3528	.5036

The variables that showed a statistically significant correlation, *P* value ≤ .05.

differences could be because these referred studies used the Papanicolaou smear staining method, whereas, in our study, the Feulgen staining method was used. According to Grover et al.,[41] the Feulgen method is considered a specific staining for DNA and MN values. They observed the lowest MN number with Feulgen compared with Papanicolaou or hematoxylin and eosin stain (H and E) and suggested the possibility of misinterpretation of nuclear anomalies and keratin granules as MN. Therefore, Feulgen staining is favored by many investigators because of its DNA specificity.[42-44]

In addition to the MN formation, other changes occur in the nucleus due to chromosomal aberrations as metanuclear anomalies, which, like MN, are also indicative of cytotoxicity and genotoxicity; these are karyolysis, karyorrhexis, binucleation, broken-eggs, and nuclear bud. Its origin may arise from an injury or cell death, in addition to defects during cell division.[42,45] In this study, the hookah group presented a higher frequency of broken eggs than cigarette smokers, which was contrary to the Bohrer et al.[36] that observed the highest broken egg in the control group, suggesting that this alteration may be associated with the DNA repair.

The karyolysis prevalence associated with consumption time in years in cigarette smokers, herein, may be explained by the cumulative effect of carcinogens on the oral mucosa. This is supported by Nersesyan et al.[46] who highlighted that the MN induction, binucleation, karyorrhexis, and karyolysis increased significantly with daily exposure to tar. Additionally, the karyorrhexis presented a positive correlation with alcohol use only in the cigarette group, similar to the study of Vassoler et al.,[47] which observed a significant increase of metanuclear alterations in smokers and drinker individuals. Jindal et al.[48] observed a significant increase in the number of MN in smokers who consumed alcohol and stated that cigarette consumption associated with alcohol could lead to carcinogenic alterations, given that alcohol increases the permeability of cell membranes to carcinogens.[49]

The observation of approximately 30% of both smoker groups presenting inflammatory alterations in smears and none of the individuals in the control group presenting inflammatory alterations agrees with those of Pavanello et al.,[50] who showed a higher proportion of inflammatory cells in smokers compared with non-smokers.

The observed results associated with changes in the superficial cell without nucleus variables, mucus, and keratohyalin granules agree with the reported literature that describes a cumulative effect of cigarettes on oral tissues[51] and with studies that noted the presence of intermediate cells in smokers of cigarettes with exposure of 10 years for evaluation of DNA gene expression repair,[52] and methylation of DNA repair genes.[53] The statistically significant differences between the hookah vs

Table IV. Mean and SD of NA, CA, and NA/CA ratio for the studied groups

Group	*Control*		*Cigarette*		*Hookah*	
NA*	54.03 ± 15.99	A	58.3 ± 16.31	B	57.36 ± 7.01	B
CA*	1671.12 ± 220.91	A	1586.95 ± 175.17	A	1580.00 ± 214.31	A
NA/CA*	0.03 ± 0.01	A	0.04 ± 0.01	B	0.04 ± 0.01	B

*Unit μm^2.
A and B represent statistically significant differences or not.
NA, nuclear area; *CA*, cytoplasmic area.

Table V. Distribution of *P* value in relation to the variables examined using Kruskal–Wallis test and Dunn's multiple comparison tests to assess cytomorphometry

Variables	*Kruskal–Wallis's test*	*Dunn's test*		
		Hookah group × Control group	*Cigarette group × Control group*	*Hookah group × Cigarette group*
NA*	.0020	.004	.002	.664
CA*	.433	.233	.342	.993
NA/CA*	.0022	.006	.001	.485

*Unit μm^2.
A and B represent statistically significant differences or not. Variables with a statistically significant correlation, *P* value $\leq$ .05.

cigarette groups only in the bacterial colonies' variant may indicate a greater reduction in normal tissue protection in the cigarette group.[54]

Both tobacco consumption habits represent direct contact of the mucosa with the produced smoke, which has large amounts of toxic substances, and its consumption is associated with several malignant neoplasms.[8] In this context, the habit of smoking hookah is related to oral carcinogenesis, harming the oral mucosa, in a similar way to the habit of smoking cigarettes.[55]

Exfoliative cytology in conjunction with cytomorphometry in this study indicates that these tools can help in monitoring clinically suspicious lesions and in early detection of malignancy in high-risk individuals, such as smokers, as also noted by Udayashankar et al.[56] Cytomorphometry uses quantitative parameters such as NA, CA, and the NA/CA ratio to assess cellular changes, because basic defects or cellular alterations start at the molecular level and can trigger a series of reactions that in turn affect the entire cellular system and, consequently, the cell morphology.[56]

The significant increase in the NA in smoker groups in the present study coincides with those of Jain et al.[57] and Srilatha et al.,[58] who also observed a significant increase in NA in smokers and users of chewable tobacco compared with their control groups. Göregen et al.[59] attributed this increase in NA to the occurrence of a cellular adaptation related to smoking, and they discussed that in this adaptive process of the nucleus, there is a tendency for dysplastic cell formation.

The observed decrease in CA, which was not significantly different for any of the 3 groups, is similar to the findings by Ogden et al.[60] and Batista et al.,[61] who did not observe significant changes in CA in young individuals who smoked cigarettes. They explained that the findings indicated the need for prolonged exposure time for cellular changes to occur. The CA decrease may be an early indicator of dysplastic change.[58,62]

The results of the NA/CA ratio in hookah and cigarette groups were significantly higher compared with the control group; however, there was no significant difference between the cigarette and hookah groups. This observation follows the studies conducted by Singh et al.[63] and Jain et al.,[57] which suggest that adaptive changes in the cell nucleus tend to be a progression toward dysplastic changes. Only in the cigarette group the NA/CA ratio was influenced by the time of consumption, probably due to the longer exposure to tobacco than the hookah group because the average consumption time in the cigarette group was 3 times greater than the hookah group.

Kokila et al.[64] associated cytomorphometry with the MN test to evaluate the tobacco's effect on tobacco smokers, tobacco chewers, and combined habit groups. Both methods of assessment revealed genetic damage in the oral mucosa in different forms of consumption, especially in combined tobacco users. Such damage was associated with a higher number of MN, with an increase in NA and NA/CA and a decrease in CA. The authors demonstrated that tobacco in any form of consumption has a genotoxic effect, and likewise, in our study, the results were similar, associating methods such as the MN test, cytomorphometry, and morphologic and tissue maturation evaluation.

Furthermore, a significant association was observed between NA alterations and the frequency of alcohol consumption in the cigarette group; this frequency and the risk related to its consumption were assessed using the AUDIT test. These results are in agreement with the literature that describes alcohol as a strong potentiating agent in the onset of malignant lesions[65,66] because ethanol present in alcoholic beverages acts as a solvent on the oral mucosa and increases the cell membrane permeability to carcinogens.[67,68] The associated use of alcohol and tobacco, in rolling or chewing form, significantly increases the chances of occurrence of oral cancer,[69] and the risk is higher with the increase of alcohol consumption.[70] A broad prevalence study carried out with 21,160 individuals with oral cancer corroborates these findings and emphasizes that the concomitant use of alcohol and tobacco contributes to a higher prevalence of advanced cases of oral cancer.[71]

According to the current findings, hookah and cigarette users present similar cytogenetic damage, as evidenced by the high frequency of karyolysis and binucleation metanuclear anomalies. However, they did not demonstrate a high frequency of MN, as

typically noted in other studies. The Papanicolaou analysis showed a cytotoxic effect of smoking tobacco, such as alterations in cell maturation and inflammatory and morpho-pathologic cells. In addition, the cytomorphometric analysis revealed important nuclear alterations in both groups of smokers, and its synergistic use with alcohol seems to promote more detrimental effects. In summary, hookah and cigarettes presented similar effects on the epithelial cells, as both promoted significant metanuclear, cytomorphometric, and morphologic changes, as well as cellular maturation, representing substantial cytogenetic damage. An additional validation to expand the scope and address some limitations of this study would be to recruit groups with a larger number of participants, investigate the use of hookah for a longer period of time (comparable to cigarettes), and/or carry out a more detailed consideration of the association of smoking with alcohol.

The present study explored non-invasive forms of analysis through the use of exfoliative cytology combined with other diagnostic methods (MN, cytomorphometry, and Pananicolaou method) as a tool for the early evaluation of oral carcinogenesis associated with different forms of tobacco consumption, demonstrating that these assessments can contribute toward diagnosis. It is important to emphasize that the gold standard diagnosis of oral cancer is biopsy with histologic evaluation. Furthermore, the results presented herein allow us to conclude that hookah and cigarettes presented similar effects in terms of cytogenetic and cytotoxic damage to oral epithelial cells.

PRESENTATION

We declare that this study has already been presented at the 48° Congresso Brasileiro de Estomatologia e Patologia Oral, 2023. The abstract will be published in Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology.

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DISCLOSURE

None.

SUPPLEMENTARY MATERIALS

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