ORIGINAL RESEARCH

Evaluation of the Genotoxicity of Tobacco and Alcohol in Oral Mucosa Cells: A Pilot Study

Tauane Vassoler[1], Letícia C Dogenski[2], Vanessa K Sartori[3], Julia S Presotto[4], Moisés Z Cardoso[5], Julia Zandoná[6], Micheline S Trentin[7], Maria SS Linden[8], Huriel S Palhano[9], Jose E Vargas[10], João P De Carli[11]

Abstract

Aim and objective: To define the genotoxic potential of tobacco and alcohol in the oral mucosa through a micronuclei (MN) test.

Materials and methods: Samples of exfoliative cells from oral mucosa were collected using superficial scraping of the right- and left-cheek mucosa of 83 patients divided into four groups, namely: (G1) 24 individuals abstaining from tobacco and alcoholic beverages; (G2) 23 individuals who smoke and abstain from alcoholic beverages; (G3) 24 smokers and alcoholics; and (G4) 12 individuals who consume alcohol and abstain from tobacco. The samples were stained with Giemsa-Wright, and the frequencies of MN, binucleated cells, and metanuclear changes were recorded in the samples of each group (1,000 cells per patient).

Results: Analysis of variance (ANOVA) showed a difference between groups for changes concerning karyorrhexis ($p = 0$), pycnosis ($p = 0.002$), karyolysis ($p = 0.003$), and binucleated cells ($p = 0.046$). As for the total number of changes, G3, G2, and G4, respectively, were significantly higher than G1.

Conclusion: It is suggested that the influence of smoking and drinking on exfoliating cells of oral mucosa may cause metanuclear changes due to genetic changes that these products cause, and the MN test is effective in detecting and monitoring such changes.

Clinical significance: MN test may work for constantly monitoring the oral mucosa of smokers and/or alcoholic patients, so that early cell changes may be diagnosed, preventing the genesis of oral cancer.

Keywords: Alcoholism, Genotoxicity, Micronuclei, Oral cancer, Smoking.

The Journal of Contemporary Dental Practice (2021): 10.5005/jp-journals-10024-3145

Introduction

Oral cancer has been observed as a multifactorial disease whose etiology involves environmental, genetic, and epigenetic factors,[1] which may occur anywhere in the mucosa, especially on the lips, floor of the mouth, and tongue. The incidence rates of oral cancer vary extensively worldwide due to the adaptation of social behaviors, such as alcoholism, smoking, betel quid chewing, and using smokeless tobacco.[1] It is reported that 75% of oral cancers are related to the lifestyle choices of patients.[2] These external carcinogenic factors cause cancer by inducing DNA damage and abnormalities in genetic processes, which accumulate in cells and lead to their degeneration.[1]

It has long been accepted that consuming tobacco and alcohol, which have a synergistic effect, is the main etiological factors for developing oral cancer,[3] even though the associated molecular mechanisms are not fully understood. Tobacco smoke includes more than 4,000 chemical substances, and at least 60 of them are carcinogens, such as nicotine. Moreover, tobacco consumption generates free radicals that reduce the antioxidant property of saliva and creates a prooxidant environment in the oral cavity.[2,4] Alcohol, in turn, increases the activation of procarcinogens and behaves as a solvent for the entry of harmful carcinogens into the body's cells. It may also contain volatile or nonvolatile flavor compounds and impurities with carcinogenic potential.[2,5]

Oral and respiratory epithelial cells, once exposed to all mutagenic components of cigarettes, alcohol, and other toxic chemicals, and for constituting the first physical barrier exposed to these genotoxic agents, are excellent for monitoring exposed populations and surveying nuclear changes in cells before the clinical symptoms of cancer appear.[6–8] In human populations, biomonitoring studies are a useful tool for assessing genetic risks from exposure to chemicals, considering these biomarkers have been associated with a high cancer risk, aging, and neurodegenerative disorders.[9]

Micronuclei (MN) are small separate nuclei added to the main nucleus that arise during mitosis of the basal layer of the epithelium as an extrachromosomal particle resulting from

[1,10]Institute of Biological Sciences, University of Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil

[2]Postgraduate Program in Oral Diagnosis of the Faculty of Dentistry, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

[3–5,7–9,11]Postgraduate Program in Dentistry, University of Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil

[6]Postgraduate Program in Dentistry, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Corresponding Author: João P De Carli, Postgraduate Program in Dentistry, University of Passo Fundo, Passo Fundo, Rio Grande do Sul, Brazil, e-mail: joaodecarli@upf.br

How to cite this article: Vassoler T, Dogenski LC, Sartori VK, *et al.* Evaluation of the Genotoxicity of Tobacco and Alcohol in Oral Mucosa Cells: A Pilot Study. J Contemp Dent Pract 2021;22(7):745–750.

Source of support: Nil

Conflict of interest: None

© Jaypee Brothers Medical Publishers. 2021 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and non-commercial reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

aberrant mitoses, when chromosomal fragments or whole chromosomes are lost or are not included in the nuclei of the daughter cells, thus remaining in the cytoplasm without any structural connection with the main core. Since the oral epithelium is maintained by continuous cell renewal, new cells produced in the basal layer by mitoses migrate to the surface, replacing those that are eliminated. The daughter cells end up differentiating in the cell layer of the spinous stratum and the keratinized superficial layer and then exfoliate in the oral cavity, providing a comprehensive assessment of genome damages caused by exposure to genotoxic substances.[6,8] The detection of micronucleated cells in the epithelial tissue is considered indicative of cancer risk for representing chromosomal damage, which is a characteristic of the initiation phase of carcinogenesis.[10]

Suitability in field conditions (noninvasiveness of the procedure, easy preparation of the slides, simple storage, and easy transport at room temperature), speed, and low cost support the oral MN cytome assay as a useful and appropriate technique.[10,11] Thus, the present study aimed to assess the genotoxic potential of tobacco and alcohol in exfoliative cells of the oral mucosa in a series of 83 patients.

Materials and Methods

Ethical Considerations

This study was approved by the Research Ethics Committee of the University of Passo Fundo (N° 2.247.544/2017). The volunteers involved in this research filled out and signed an informed consent form.

Selection of Patients

The present study is a cross-sectional observational laboratory experiment since it evaluated the cellular characteristics that indicate genotoxicity [MN and metanuclear alterations (MA)] of individuals in a single moment of time, comparing them to the type of addiction (smoking and/or alcoholism). The samples were collected at the School of Dentistry of the University of Passo Fundo, in the city of Passo Fundo/RS, Brazil, and analyzed at the Cell Biology Laboratory of the Institute of Biological Sciences at the University of Passo Fundo, Passo Fundo/RS, Brazil. Through a preliminary analysis of 2,426 clinical records, 83 patients who met the inclusion and exclusion criteria of the study were selected.

The inclusion criteria consisted of patients between 30 and 50 years of age, of both sexes, who did not use any continuous medication and did not present current or previous systemic diseases.

The patients excluded from the study had contact with potentially genotoxic agents other than those of interest for the research (e.g., farmers and workers in the chemical industry or gas stations) or had lesions in the oral region that could interfere with the cell analysis results.

The patients selected were divided into four groups:

G1: 24 individuals who had never used tobacco and abstained from alcoholic beverages;

G2: 23 individuals who smoked at least 10 cigarettes daily for at least 1 year and abstained from alcoholic beverages;

G3: 24 individuals who smoked at least 10 cigarettes daily for at least 1 year and consumed an equivalent of 60 g or more of alcohol/week (six glasses of wine, six glasses of beer, or six doses of a distilled beverage) for at least 1 year;

G4: 12 individuals who abstained from tobacco and consumed 60 g or more of alcohol/week (six glasses of wine, six glasses of beer, or six doses of a distilled beverage) for at least 1 year.

The number of patients included in each group was different since the sample studied was of convenience.

Sample Collection and Analysis

The cells were collected using superficial scraping of the right- and-left cheek mucosa, aided by a disposable wooden spatula. The material collected was deposited in vials containing 3:1 methanol and acetic acid fixing solution, transferred to a Falcon tube, and centrifuged at 1,000 rpm for 10 minutes. This process was repeated until the material was colorless and free of residues.

After cleaning, the material was dripped onto histological slides that were stained with Wright-Giemsa. In these stains, the components are oxidized methylene blue, azure B, and eosin Y dyes. The methylene blue and azure B dyes stain the nucleus varying from blue to purple, and the eosin Y dye stains the cytoplasm of the cells from an orange to pink color.

For each individual, 1,000 cells of the oral mucosa were analyzed under an optical microscope (Olympus Bx50, Tokyo, Honshu, Japan), at a magnification of 400×. An experienced, trained, and blinded examiner performed the analysis. The Kappa test was used to assess the examiner's level of agreement ($k = 0.90$).

The criteria used for scoring MN and MA were according to those described by Thomas et al.[12] The results are presented as the number of cells with MN and MA per 1,000 cells. The abnormalities were evaluated by assessing the staining intensity, texture similar to nucleus, focal plane of the nucleus, and rounded smooth perimeter suggestive of a membrane. Normal cells were identified as follows: Intact and relative homogeneous cytoplasm, little or no contact with adjacent cells, and an intact homogenous nucleus with a smooth and distinct nuclear perimeter.[12,13]

The amount of observed MN and MA, including binucleated cells, nuclear buds, nuclear alterations type 1 (karyorrhexis), 2 (pycnosis), and 3 (karyolysis), was recorded according to previous studies.[12–14] Micronucleated cells were characterized by the presence of the main nucleus and a smaller one, called MN, resulting from a chromosomal fragmentation by genotoxicity. The MN cells were characterized according to the following criteria: (a) Regular contour, round, or elliptical; (b) similar staining to the main nucleus; (c) less than one-third of the diameter of the nucleus; and (d) completely separated from the nucleus, allowing clear identification between the nucleus and MN limits. The overlapping cells were excluded; karyorrhexis cells were characterized by more extensive chromatid aggregation indicating fragmentation and nuclear disintegration in the advanced stage of cell death by apoptosis; pycnotic cells were characterized by a small nucleus with condensed chromatin and intense staining. Nuclear diameter is 1/3 to 2/3 smaller than that of the differentiated cells and is related to an advanced stage of cell death by necrosis; karyolysis cells have a lightly stained chromatin that is difficult to be analyzed under light microscopy and related to a more advanced stage of cell death process due to necrosis; binucleated cells were characterized by the presence of two nuclei with characteristics similar to

JAYPEE

differentiated cells. The presence of binucleation is indicative of failure due to cytotoxic action in the cytokinesis process during cell reproduction; bud cells showed the main nucleus and the nearby accessory core connected by fine chromatin filaments. The accessory core has the same morphological and coloring characteristics as the main core. However, it has a diameter less than 1/4 of the core. It is believed that this type of morphology originates from the presence of dicentric chromosomes with abnormal anaphasic behavior during segregation (Fig. 1).

Statistical Analysis

Levene and Shapiro-Wilk tests were used to check homogeneity and normal sample distribution ($p > 0.05$). The statistical analysis was performed with ANOVA and Tukey's tests at a 95% significance level.

Results

ANOVA (Table 1) showed a difference between groups for changes concerning karyorrhexis ($p = 0.000$), pycnosis ($p = 0.002$), karyolysis ($p = 0.003$), and binucleated cells ($p = 0.046$). Tukey's post hoc showed a higher number of changes of the karyorrhexis type for G3, composed of smokers and alcoholic patients ($p = 0$).

The cases of pycnosis and karyolysis were also statistically higher when comparing G3 to the control group (G1) and the group composed only of alcoholic patients (G4) ($p < 0.05$). For binucleated cells, the number of changes was also higher in G3 than in G1. As for the total number of changes, G3, G2, and G4, respectively, were significantly higher than G1 (Table 2).

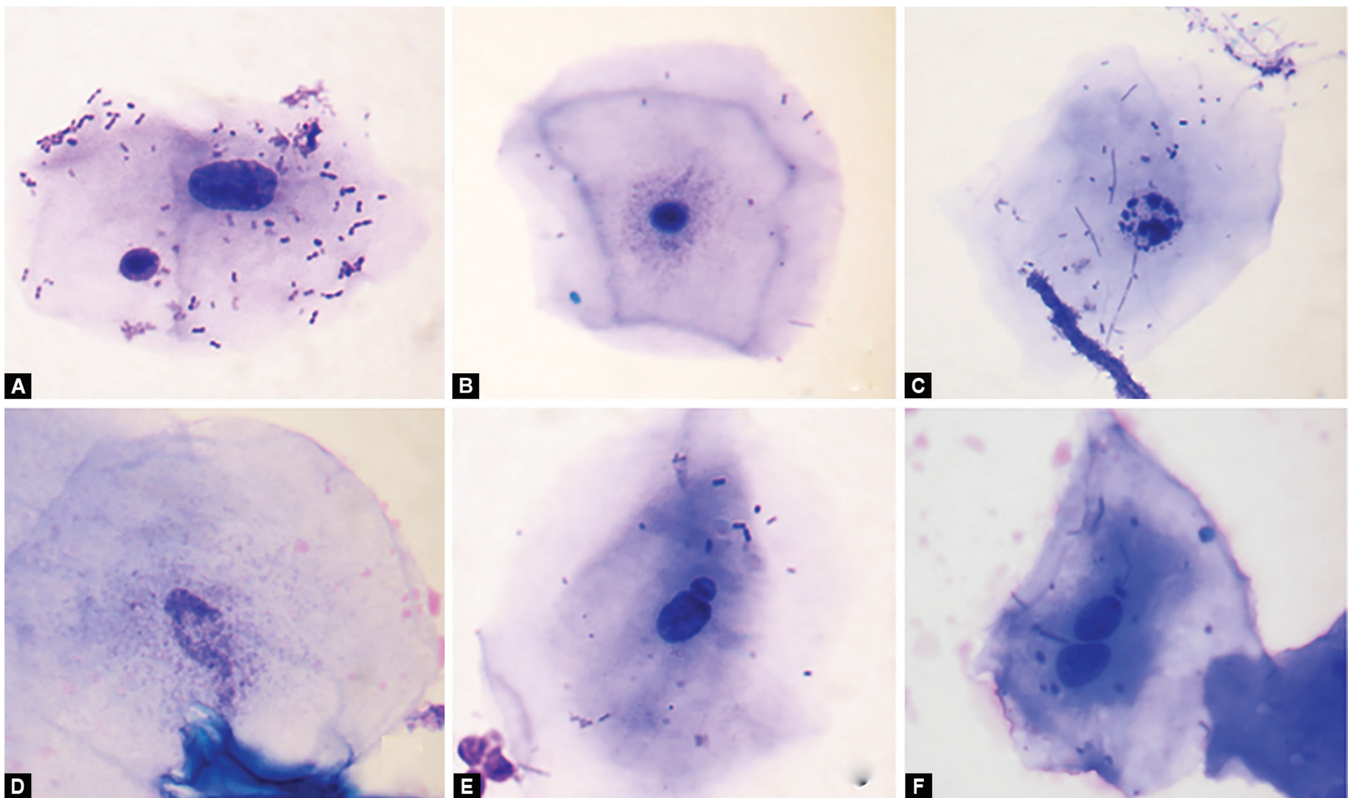
The total number of changes, mainly karyorrhexis and karyolysis, was higher in patients who smoked more than two packs of cigarettes a day in G3 (Table 3).

Based on the results obtained in this study, it can be inferred that there is a considerable genotoxic potential of tobacco and alcohol in the oral mucosa (especially when associated). Thus, it is noted that the methodology employed in this study (MN test) is suitable for constant monitoring of the oral mucosa of smoking and/or alcoholic patients.

Discussion

The results obtained with the present study confirm the hypothesis that genetic changes may be induced by the presence of genotoxic agents from tobacco and alcohol, which corroborates information previously reported in the literature.[15–17] The MN assay was used in this study for representing a minimally invasive and low-cost method for studying DNA damage, chromosomal instability, cell death, and the regenerative potential of human oral mucosa tissue. Thus, it is suggested that the MN study may work, especially in the public health system, as a constant monitoring test of the oral mucosa of smokers and alcoholic patients so that early cell changes can be diagnosed, preventing the genesis of oral cancer.

According to Rana et al.,[18] the prevalence of MN in a healthy population was reported as 0–0.9%, and the increased number of such structures would represent chromosomal aberrations. Tomiazzi et al.[17] highlight that the increased incidence of MN in exfoliated cells of the oral mucosa has been related to occupational



Figs 1A to F: Cells of the oral mucosa stained with Giemsa-Wright: (A) Micronucleus; (B) Pycnosis; (C) Karyorrhexis; (D) Karyolysis; (E) Bud; (F) Binucleated

Table 1: Mean (SD) of the change values according to the groups studied. Indication of the results obtained by ANOVA comparing the study groups

Change	*Groups*	*n*	*Mean*	*SD*	*Difference/ statistical similarity*
Karyorrhexis $p = 0.000$	Control	24	1.8333	2.68112	A
	Smokers	23	2.9565	4.70472	A
	Alcoholics and smokers	24	10.2917	9.64356	B
	Alcoholics	12	2.9167	1.83196	A
Pycnosis $p = 0.002$	Control	24	0.6250	1.27901	A
	Smokers	23	2.4783	3.08765	AB
	Alcoholics and smokers	24	4.5000	4.69968	C
	Alcoholics	12	2.0000	3.59292	AB
Karyolysis $p = 0.003$	Control	24	1.7917	1.64129	A
	Smokers	23	2.7391	4.28759	AB
	Alcoholics and smokers	24	5.1667	4.49799	B
	Alcoholics	12	1.2500	1.48477	C
Binucleated $p = 0.046$	Control	24	2.4583	2.12601	A
	Smokers	23	3.7826	3.67988	AB
	Alcoholics and smokers	24	5.4167	4.68964	C
	Alcoholics	12	4.5000	3.31662	B
Bud $p = 0.168$	Control	24	0.4167	1.13890g	A
	Smokers	23	0.6957	0.82212	B
	Alcoholics and smokers	24	1.2083	1.84106	C
	Alcoholics	12	0.5000	0.79772	D
MN $p = 0.825$	Control	24	2.4167	1.97631	A
	Smokers	23	2.5217	3.27344	A
	Alcoholics and smokers	24	3.1667	3.63158	A
	Alcoholics	12	3.0000	3.56753	A

Different letters indicate a statistical difference between the groups

Table 2: Mean (SD) of the total change values according to the groups

	Group	*n*	*Mean*	*SD*	*p*
Smokers	Control	24	9.54	5.501	0.059
	Smokers	23	15.17	13.089	
Alcoholics and smokers	Control	24	9.54	5.501	0.000
	Alcoholics and smokers	24	28.88	13.715	
Alcoholics	Control	24	9.54	5.501	0.034
	Alcoholics	12	14.17	6.7269	

Comparison between study groups for total changes (values of $p < 0.05$ indicate a statistical difference between the groups)

exposure to pesticides and smoking considering that the inclusion or exclusion criteria were strictly followed.

Regarding the oral cavity, smoking has been described as the main risk factor for developing malignant and cancerous lesions,[19] while the combination of deleterious habits, such as alcoholism and smoking, may increase the prevalence of mouth cancer. A greater aggressiveness of this disease in alcoholics and smokers may occur due to the increased permeability of the cell membrane caused by ethanol and consequent exposure of intracellular content to tobacco as a carcinogen.[20] In this study, there was no statistically significant difference in the number of MN in the four groups studied, but numerically, the group of smoking and alcoholic patients (G3) presented the highest average in the number of MN, followed by the alcoholic group (G4), smokers (G2), and the control group (G1).

This study showed a difference between the groups for the average of changes concerning karyorrhexis, pycnosis, karyolysis, and binucleated cells, always showing G3 with a statistically higher average than the other groups. Based on the findings of the present study and previous statements in the literature, the smoking and alcoholic patients analyzed in this research do not yet have a neoplastic process installed, but have a markedly increased susceptibility to the development of malignant pathologies. This finding is based on the fact that alcoholism associated with smoking induces a decrease in the expression of interleukin 18 (IL-18) and protein DDX3, which regulate the cell cycle and the progression of malignancy. In addition, the inflammatory marker COX-2, related to the aggressiveness of neoplasms, is more frequent in smokers than in nonsmokers.[20]

In the study by Upadhyay et al.,[15] which sought to analyze the genotoxic effects of tobacco in individuals with a habit of smoking and chewing tobacco, the percentage of micronucleated cells was 7.589 ± 5.672 for smokers, 10.413 ± 3.865 for individuals with the habit of chewing tobacco, 21.996 ± 9.916 for smokers and chewers, and 1.033 ± 1.2658 for the control group, which did not have smoking or chewing tobacco habits. The study concluded that there is a significant increase in the frequency of MN counting in individuals with a habit of smoking, chewing tobacco, or smoking and chewing tobacco concurrently, when compared to the control group, indicating that genotoxic and carcinogenic agents released by tobacco generate considerable genetic damage.

The results obtained in the present research showed that the total number of cellular changes, especially karyorrhexis and karyolysis, was higher in patients who smoked more than two packs of cigarettes a day in the group of smokers and alcoholics (G3) ($p < 0.05$). Besides that, the total changes were higher in individuals who consumed alcohol than in the control group ($p < 0.05$). Our results agree with those by Gutiérrez et al.,[21] which aimed to analyze and characterize the levels of volatile organic compounds (VOC), particulate matter (PM), and polycyclic aromatic hydrocarbons (PAH) in two areas of Buenos Aires: The urban area of the city of La Plata and the industrial area of Ensenada. In this study, the potential health risk of the inhabitants was evaluated through a genotoxic assay in exfoliated oral mucosa cells through MN analysis. In the results, the study showed that the smoking habit represented a significant factor for increasing the percentage of MN. Although it did not focus on assessing changes for smoking and drinking habits, this study also revealed that people exposed to VOC, PM, and PAH showed cellular changes that were potentiated in those who smoked and consumed alcoholic beverages concurrently.

A study by Tomiazzi et al.,[17] which aimed to assess the genotoxic effects of exposure to pesticides with and without combined exposure to cigarette smoke in Brazilian agricultural workers, showed that the total number of cytogenetic changes and MN was higher in all groups exposed to xenobiotics, with possible carcinogenic potential when compared to the control group. Additionally, exposure to pesticides has proven to be more harmful

Table 3: Daily amount of cigarettes (white cells) and alcohol (gray cells) in the groups of smokers (G2), smokers and alcoholics (G3), and alcoholics (G4), related to the mean and standard deviation of MN and metanuclear changes present in 1,000 epithelial cells of the oral mucosa

Group	*Number of cigarettes/day* *Amount of alcohol*	*MN* *Mean (SD)*	*Karyorrhexis* *Mean (SD)*	*Pycnosis* *Mean (SD)*	*Karyolysis* *Mean (SD)*	*Binucleated* *Mean (SD)*	*Bud* *Mean (SD)*
Smokers (G2) *n* = 23	13 patients (56.52%)—10 cigarettes/day (half a pack)	4.38 (3.30) A	2.15 (1.67) A	2.92 (3.42) A	3.15 (4.43) A	5.84 (3.46) A	0.76 (0.83) A
	10 patients (43.48%)—10–20 cigarettes/day (half to 1 pack)	0.10 (0.31) A	4.00 (6.94) A	1.9 (2.64) A	2.20 (4.26) A	1.10 (1.72) A	0.60 (0.84) A
Alcoholics and smokers (G3) *n* = 24	13 patients (54.17%)—10 cigarettes/day (half a pack)	3.46 (3.35)	**7.53 (7.32) A**	4.53 (5.88)	**5.07 (5.04) A**	4.76 (4.24)	1.76 (2.27)
	10 patients (41.67%)—350–700 mL/day	2.30 (2.45)	12.80 (10.03)	6.60 (6.11)	4.70 (3.26)	4.50 (4.90)	1.00 (1.41)
	06 patients (25%)—10–20 cigarettes/day (half to 1 pack)	3.00 (4.56)	**9.83 (10.45) A**	4.50 (4.27)	**2.00 (1.41) A**	5.33 (7.00)	0 (0)
	09 patients (37.50%)—1 L/day	2.25 (4.16)	10.87 (11.11)	2.62 (3.20)	6.00 (6.52)	4.62 (2.61)	1.75 (2.65)
	05 patients (20.83%)—>40 cigarettes/day (>2 packs)	1.60 (3.04)	**17.40 (12.50) B**	3.60 (2.07)	**8.60 (3.43) B**	5.20 (3.42)	2.00 (1.22)
	05 patients (20.83%)—>1 L/day	5.80 (3.89)	2.60 (0.89)	2.20 (2.16)	3.00 (2.34)	6.40 (7.60)	1.20 (1.64)
Alcoholics (G4) *n* = 12	03 patients (25%)—200 mL/day	2.00 (1.73)	3.67 (1.15)	5.67 (6.02)	2.67 (1.15)	5.67 (3.78)	0.33 (0.57)
	03 patients (25%)—350–700 mL/day	3.00 (2.00)	1.67 (2.08)	1.33 (2.30)	0.33 (0.57)	6.33 (3.05)	0 (0)
	06 patients (50%)—>1 L/day	3.50 (4.92)	3.16 (1.94)	0.50 (0.83)	1.00 (1.54)	3.00 (3.03)	0.83 (0.98)

than smoking. The authors conclude that this genotoxic potential is alarming, as it may indicate an increased risk of developing oral cancer in this population, also indicating the importance of biomonitoring studies in populations exposed to pesticides, with particular emphasis on agricultural workers.

The limitation of the present study concerns the fact that patients were not followed up over time regarding possible clinical manifestations or changes in the cellular alterations observed. This limitation leads to the suggestion that further clinical and laboratory longitudinal studies should be carried out.

Conclusion

The present study allows us to conclude that, when individuals are exposed to tobacco and alcohol simultaneously, cellular changes occur significantly greater than in individuals who do not use these substances. Thus, it appears that the MN test is effective in monitoring cellular changes, possibly serving as a screening method to avoid possible dysplastic clinical manifestations.

Clinical Significance

It is suggested that the MN test may work for constantly monitoring the oral mucosa of smokers and/or alcoholic patients, as well as a screening test in epidemiological studies, so that early cell changes may be diagnosed, preventing the genesis of oral cancer.

References

1. Ali J, Sabiha B, Jan HU, et al. Genetic etiology of oral cancer. Oral Oncol 2017;70:23–28. DOI: 10.1016/j.oraloncology.2017.05.004.
2. D'souza S, Addepalli V. Preventive measures in oral cancer: an overview. Biomed Pharmacother 2018;107:72–80. DOI: 10.1016/j.biopha.2018.07.114.
3. Dhanuthai K, Rojanawatsirivej S, Thosaporn W, et al. Oral cancer: A multicenter study. Med Oral Patol Oral Cir Bucal 2018;23(1):e23–e29. DOI: 10.4317/medoral.21999.
4. Ghantous Y, Schussel JL, Brait M. Tobacco and alcohol-induced epigenetic changes in oral carcinoma. Curr Opin Oncol 2018;30(3):152–158. DOI: 10.1097/CCO.0000000000000444.
5. Ogden GR. Alcohol and mouth cancer. Br Dent J 2018;225(9):880–883. DOI: 10.1038/sj.bdj.2018.921.
6. Dutta S, Bahadur M. Cytogenetic analysis of micronuclei and cell death parameters in epithelial cells of pesticide exposed tea garden workers. Toxicol Mech Methods 2016;26(8):627–634. DOI: 10.1080/15376516.2016.1230917.
7. Tomar SL, Hecht SS, Jaspers I, et al. Oral health effects of combusted and smokeless tobacco products. Adv Dent Res 2019;30(1):4–10. DOI: 10.1177/0022034519872480.
8. Farhadi S, Jolehar M, Safapour F. Micronucleus assay of buccal mucosal cells in hairdressers: the importance of occupational exposure. Asian Pac J Cancer Prev 2018;19(8):2131–2134. DOI: 10.22034/APJCP.2018.19.8.2131.
9. Cobanoglu H, Coskun M, Coskun M, et al. Results of buccal micronucleus cytome assay in pesticide-exposed and non-exposed group. Environ Sci Pollut Res Int 2019;26(19):19676–19683. DOI: 10.1007/s11356-019-05249-0.
10. Claudio SR, Simas JMM, Souza ACF, et al. Genomic instability and cytotoxicity in buccal mucosal cells of workers in banana farming evaluated by micronucleus test. Anticancer Res 2019;39(3):1283–1286. DOI: 10.21873/anticanres.13239.
11. Hutter HP, Khan AW, Lemmerer K, et al. Cytotoxic and genotoxic effects of pesticide exposure in male coffee farmworkers of the Jarabacoa region, Dominican Republic. Int J Environ Res Public Health 2018;15(8):1641. DOI: 10.3390/ijerph15081641.
12. Thomas P, Holland N, Bolognesi C, et al. Buccal micronucleus cytome assay. Nat Protoc 2009;4(6):825–837. DOI: 10.1038/nprot.2009.53.
13. Bolognesi C, Knasmueller S, Nersesyan A, et al. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay - an update and expanded photogallery. Mutat Res 2013;753(2):100–113. DOI: 10.1016/j.mrrev.2013.07.002.
14. Bonacina LV, Vanini J, Zandoná J, et al. Genotoxicity of 22% carbamide peroxide bleaching agent on oral cells using the micronucleus technique. J Clin Diagn Res 2020;14(3):ZC14–ZC17. DOI: 10.7860/JCDR/2020/43341.13569.

15. Upadhyay M, Verma P, Sabharwal R, et al. Micronuclei in exfoliated cells: A biomarker of genotoxicity in tobacco users. Niger J Surg 2019;25(1):52–59. DOI: 10.4103/njs.NJS_10_18.
16. Singam PK, Majumdar S, Uppala D, et al. Evaluation of genotoxicity by micronucleus assay in oral leukoplakia and oral squamous cell carcinoma with deleterious habits. J Oral Maxillofac Pathol 2019;23(2):300. DOI: 10.4103/jomfp.JOMFP_221_19.
17. Tomiazzi JS, Judai MA, Nai GA, et al. Evaluation of genotoxic effects in Brazilian agricultural workers exposed to pesticides and cigarette smoke using machine-learning algorithms. Environ Sci Pollut Res Int 2017;25(2):1259–1269. DOI: 10.1007/s11356-017-0496-y.
18. Rana SVS, Verma Y, Singh GD. Assessment of genotoxicity amongst smokers, alcoholics, and tobacco chewers of North India using micronucleus assay and urinary 8-hydroxyl-2′-deoxyguanosine, as biomarkers. Environ Monit Assess 2017;189(8):391. DOI: 10.1007/s10661-017-6103-3.
19. Chaturvedi P, Singh A, Chien CY, et al. Tobacco related oral cancer. BMJ 2019;365:l2142. DOI: 10.1136/bmj.l2142.
20. Bezerra NV, Leite KL, de Medeiros MM, et al. Impact of the anatomical location, alcoholism and smoking on the prevalence of advanced oral cancer in Brazil. Med Oral Patol Oral Cir Bucal 2018;23(3):e295–e301. DOI: 10.4317/medoral.22318.
21. Gutiérrez MLA, Palmieri MA, Giuliani DS, et al. Monitoring human genotoxicity risk associated to urban and industrial Buenos Aires air pollution exposure. Environ Sci Pollut Res Int 2020;27(12): 13995–14006. DOI: 10.1007/s11356-020-07863-9.