Original Research
Oral Pathology

Genotoxicity in the oral cells of older people from a Brazilian rural area: a population-based study

Luana Soares KUZE[a]
João Paulo DE CARLI[a]
Júlia Stephanie PRESOTTO[a]
Kaue COLLARES[a]
Alvaro DELLA BONA[a]

(a) Universidade de Passo Fundo – UPF, Postgraduate Program in Dentistry, Passo Fundo, RS, Brazil.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Corresponding Author:
Alvaro Della Bona
E-mail: dbona@upf.br

https://doi.org/10.1590/1807-3107bor-2023.vol37.0067

Submitted: May 22, 2022
Accepted for publication: February 27, 2023
Last revision: March 30, 2023

Abstract: The purpose of this population-based, observational, and cross-sectional study was to evaluate alterations in the oral cells of a population of older people from a Brazilian rural area, using the micronucleus technique to investigate possible associated genotoxic factors. A questionnaire was applied and clinical examination and collection of oral mucosal cells were performed for all older people (≥ 60 years) from a town in southern Brazil. Demographic and socioeconomic variables, deleterious habits (drinking and tobacco use), presence of gastro-oesophageal reflux disease (GERD), and the use of proton pump inhibitors (PPIs) were considered the exposure variables, whereas metanuclear changes (MCs) and the prevalence of cell micronuclei (MN) were considered outcomes. Out of 489 older people, 447 were included in the study, among whom 50.8% were men with a mean age of 70.9 years and 83.9% had a monthly family income greater than US$ 500.00. GERD symptoms were present in 36.2% of the individuals, and 29.1% used PPIs daily, 53.3% consumed alcoholic beverages, and 46.7% used tobacco. The analysis of 1,000 oral mucosal cells per subject showed a MN frequency of 0–2 per individual, and MCs were detected with an average of 15 units per individual (median = 11 per individual). Poisson regression did not show statistical association between the exposure variables and the outcomes (presence of MN and MCs), except for the use of PPIs, which was a protective factor for the prevalence of MN [PR 0.6 (CI 0.3–0,9)]. Age, sex, family income, tobacco use and drinking, and GERD were not associated with the number of MN and MCs in oral mucosal cells of the investigated older people.

Keywords: Risk Factors; Cell Nucleus; DNA Damage; Aged.

Introduction

Genotoxic changes in oral mucosal cells may occur because of several factors and result in DNA damage, leading to the development of dysplastic or tumour processes and, therefore, the constant monitoring of populations is essential.[1] External carcinogenic factors, either physical or chemical, cause abnormalities in genetic processes, accumulating in the cells and leading to their degeneration.[1]

Approximately 15% of the Brazilian population lives in rural areas.[2] Despite growing concerns, few studies have focused on the health of

rural populations.[3] It was reported in 2010 that oral health was the fifth most important health topic for residents of rural areas in the United States, outranked by access to good-quality health care, cardiovascular diseases, diabetes, and mental health.[4]

Older people in the Brazilian rural context have not been sufficiently investigated, perhaps because they are a minority (estimated at 15.7%) when compared to older people in the urban areas (84.3%) or because of the lack of specific public policies for this population.[5,6] Regarding the health and health care of older people living in rural areas, some difficulties are expected due to characteristics that are inherent to the access to health care (e.g., transportation barriers, poor road conditions or lack of roads, and distance from health centers), to low income, and to the tradition of seeking curative/therapeutic care rather than preventive care. Because of that, the health status and quality of life of these older people may eventually worsen.[7-9]

Cancer is one of the most common causes of morbidity and mortality and it results from the interaction of risk factors that affect the monitoring processes of cell proliferation in any region of the body.[10] Drinking and tobacco use are the main risk factors for oral cancer.[11,12] Moreover, studies have suggested that individuals with gastroesophageal reflux disease (GERD) may develop ulcerated lesions in oral tissues.[13,14]

The Union for International Cancer Control[15] considered longer life expectancy and estimated the incidence of cancer to be 11 million in 2002, increasing to more than 15 million in 2020. The incidence of oral cancer also rose, representing the sixth most common tumour worldwide, with about 40,000 cases reported every year in the United States, among which 75% are related to the environment and lifestyle[16]. Another study also reported that people's lifestyle and exposure to environmental factors may cause deleterious effects on health, including DNA damage.[17] In addition, relevant biological factors such as age, sex, use of medication, and systemic diseases may have a significant influence on DNA mutations.[18]

There are multiple tests to biologically monitor human populations exposed to mutagens. The assessment of genotoxicity using the micronuclei (MN) test in exfoliated cells is an effective and low-cost procedure to investigate epithelial carcinogens and may be used to detect chromosome breakage or mitotic interference, which plays a significant role in carcinogenesis.[19,20] MN originate from chromosomes or from their fragments and remain in the anaphase during nuclear division. The correlation between the frequency of MN/MC and the severity of this genotoxic damage has been shown.[19,20] Therefore, it may be argued that MN and MC changes in exfoliated oral epithelial cells represent important signs of early genotoxic events induced by carcinogenic agents. The present study evaluated oral cellular alterations in a population of older people from a rural area in southern Brazil, using the micronucleus technique to investigate possible associated genotoxic factors. It was hypothesized that deleterious habits (drinking and tobacco use), the presence of GERD, and continuous use of some medications – proton pump inhibitors (PPIs) – affect the number of MN and MCs in the oral epithelial cells of this population.

Methodology

The present study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.[21]

Study design and ethical considerations

This is a population-based, observational, and cross-sectional study. The research project was approved by the local Research Ethics Committee and registered in the Brazilian Platform (number 3.315.733). The older people aged over 60 years were invited to participate and were included in the study after signing an informed consent form.

Context and participants

The present study was performed in a rural town with 1,941 inhabitants located in the northeastern region of the state of Rio Grande do Sul, in southern Brazil. Approximately 25% of the population is composed of older people (age ≥ 60 years), and agriculture is the main economic activity. The data were collected between May and September 2019.

The local Health Department provided the list of inhabitants with their respective ages and addresses. The town's entire older population (≥ 60 years) was invited to participate. Data were collected from all participants at their homes on a previously scheduled date. Individuals who were not at home after two visits were regarded as losses. Moreover, individuals with mental or physical disorders who did not collaborate with the application of the questionnaires or clinical examination were excluded. Samples with less than 1,000 cells were also excluded from the study. Losses and refusals were constantly assessed so that the study would maintain a uniform and homogeneous distribution of participants per microregion. To optimize such distribution and the participation of the older people, visits were scheduled to the best possible time for the participant, avoiding any conflict with any appointment they had already scheduled.

Data sources and variables

The study variables were divided into three blocks:

a. A: demographic and socioeconomic variables, including personal data, birth date, sex, and family income;
b. B: behavioural factors, including tobacco use and drinking, which were investigated using the WHO ASSIST instrument;[22]
c. C: general health status, the use of PPIs, and gastric problems investigated by the GERD-SQ questionnaire.[23]

The present study used the following as exposure variables: information on sex (male or female), age (60 to 65 years, 66 to 74 years, and ≥ 75 years), and socioeconomic level as per the monthly family income (< US$ 500.00 and ≥ US$ 500.00). The amount (US$ 500.00) corresponds to the income of a couple receiving social security benefits such as retirement, and it is equivalent to approximately two minimum wages.

Tobacco use and drinking were measured with the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) questionnaire.[22] This eight-question instrument assesses the consumption pattern of toxic substances, such as alcohol and tobacco throughout life and especially over the last three months, classifying the individual as having low, moderate, or high risk for each substance.

GERD was also investigated as an exposure variable. It was deemed present when the participant reported a medical diagnosis of the disease or had a score ≥ 2 in questions 1 (heartburn) or 3 (regurgitation) in the GERD-SQ questionnaire.[23] This variable was categorized as either presence or absence. The daily use of PPIs was also considered an exposure variable (yes/no). In addition, the sum of three relevant factors (drinking, tobacco use, and presence of GERD) was assessed as follows: 0 = presenting none of these factors; 1 = presenting at least one of these factors; 2 = presenting at least two of these factors; 3 = presenting the three factors.

Finally, an intraoral examination was performed to collect oral mucosal cells by scraping the right and left buccal mucosa using a disposable wooden spatula. The collected material was placed in flasks containing a fixative solution of acetic acid and methanol (3:1) until processing of the sample.[24] Prior to cell collection, the participants rinsed their mouths with 10 mL of distilled water for 1 min to remove possible food residues or dead cell debris from the buccal mucosa, thus avoiding any interference with the interpretation of the results.

Cell material processing

Collected oral cell samples were transferred to Falcon tubes and centrifuged at 1,000 rpm for 10 min (Ls4 centrifuge, CELM™, São José dos Campos, SP, Brazil). The process was repeated until the material was colourless and free of residues. The clear samples were then seeded onto plates stained with 10% Giemsa Wright (Renylab Química™, Barbacena, Brazil). One thousand oral mucosal cells per individual were analyzed under a light microscope (Olympus Bx50, Tokyo, Japan; magnification: x1000). An experienced, trained, and blinded examiner performed all the analyses. The kappa coefficient was used to assess the intra-examiner level of agreement (k = 0.90).

Thomas et al.[25] described the criteria used to report on the presence of MN and MCs. The results were presented as the absolute number of cells with MN and MCs found in every 1,000 cells. Cell abnormalities were considered by assessing the intensity of color, texture, and focal plane of the

nucleus. Normal cells were those that presented an intact and relatively homogeneous cytoplasm, little or no contact with adjacent cells, and an intact and homogeneous nucleus with a smooth and distinct nuclear perimeter.[24,26,27]

The number of MN and MCs was recorded.[24,26-28] Micronucleated cells were characterized by the presence of the main nucleus and a smaller one, called micronucleus (MN), resulting from chromosome breakage caused by genotoxicity.[26-28] MN evaluation also considered the presence of a) a regular round or an elliptical contour within the cytoplasm; b) similar colour to that of the main nucleus; c) less than 1/3 of the nucleus diameter; and d) complete separation from the nucleus, allowing clear identification of the nucleus and the limits of the MN. Overlapped cells were excluded from the evaluation.

Karyorrhectic cells were characterized by a more extensive chromatid aggregation, indicating nuclear fragmentation and disintegration, in an advanced stage of cell death by apoptosis. Pyknotic cells were characterized by a small nucleus with condensed chromatin and intense color. The nuclear diameter is 1/3 to 2/3 smaller than that of differentiated cells and indicates an advanced stage of cell death by necrosis. Karyolytic cells present a slightly stained chromatin, difficult to analyse under a light microscope, and indicate a more advanced stage of cell death by necrosis. Binucleated cells were characterized by the presence of two nuclei with similar characteristics to those of differentiated cells. The presence of binucleation is indicative of failure by cytotoxic action in the cytokinetic process during cell reproduction. Button cells show the main and accessory nuclei close to each other and connected by thin chromatin threads. The accessory nucleus has the same morphological characteristics and color as those of the main nucleus. However, it has a smaller diameter (1/4 of the nucleus). This type of morphology might originate from the presence of dicentric chromosomes with abnormal anaphase behaviour during segregation.[24,26-28]

The MN per 1,000 cells and the MCs per 1,000 cells were considered outcome variables, categorized as follows: MN (NO = 0; YES = 1–2) and MC dichotomized by the median (≤ 11 MC and > 11 MC).

Data collection

The application of the WHO-ASSIST questionnaire, the clinical examination, and oral cell collection were performed at the participant's home. The participant remained seated in a comfortable chair with a headrest for the oral examination and cell collection. A headlamp was used by the examiner to illuminate the oral cavity. Adequate disposable personal protective equipment (PPE – laboratory coat, goggles, gloves, mask, face shield, and cap) was used by the professionals, in addition to disposable wooden spatulas and sterile flasks containing acetic acid and methanol (3:1). The data were collected by two professionals: a dentist (examiner) and an oral health assistant (annotator). Oral cell samples were processed and evaluated by a calibrated microbiologist.

Sample size

All town residents (≥ 60 years) were invited to participate. The exclusion criteria were applied during the first visit for data collection.

Calibration, training, and pilot study

A 16-hour training lesson was used to instruct, train, and calibrate the research team. A pilot study was then performed with 20 individuals from a long-stay institution for older people in another southern Brazilian town. The pilot study helped evaluate the research protocol, adjusting the data collection instruments and method and identifying the most suitable approach for the study population. The intra-examiner agreement was obtained during the pilot study ($k = 0.90$).

Statistical analysis

A descriptive analysis was initially performed to determine the relative and absolute frequencies of exposure and outcome variables. Poisson regression analysis was used to evaluate factors associated with the presence of MN and the presence of more than 11 MCs. Prevalence ratios were obtained for the exposure variables and their respective 95% confidence intervals. All analyses were performed using the Stata software (StataCorp. 2015, Stata Statistical Software: Release 14. College Station, USA: StataCorp LP).

Results

Among those individuals who were eligible for data collection (489), 42 were excluded because they were either not at home at the scheduled time for the visits (34) or the oral mucosal cell samples did not have the minimum amount of 1,000 cells (8). Thus, 447 individuals participated in the study, with a response rate of 91.4%. The mean age of the participants was 70.9 years, 50.5% were male, and most of them had a monthly income greater than US$ 500.00 (83.7%).

Table 1 presents the distribution of covariates according to the presence of MN. Almost 20% of the participants showed at least one MN change per 1,000 cells. No participant showed more than two MN changes. Poisson regression did not show statistical association between exposure variables and outcomes, except for the use of PPIs, which was a protective factor for the prevalence of MN [PR 0.6

Table 1. Descriptive analysis and Poisson regression (PR) with confidence interval (CI) based on the dichotomized variable "presence of micronuclei" (MN) (n = 447 individuals).

Variable	MN		PR (CI)	p–value
	NO	YES		
	361 (80.7%)	86 (19.2%)		
Sex				0.93
Male	183 (80.6)	44 (19.4)	Ref	
Female	178 (80.9)	42 (19.1)	0.9 (0.6–1.4)	
Age (in years)				0.66
60–65	116 (78.3)	32 (21.6)	Ref	
66–74	121 (81.7)	27 (18.2)	0.8 (0.5–1.3)	
75 or older	124 (82.1)	27 (17.8)	0.8 (0.5–1.3)	
Family income				0.70
< US$ 500.00	57 (79.1)	15 (20.8)	Ref	
≥ US$ 500.00	304 (81.0)	71 (18.9)	0.9 (0.5–1.4)	
Drinking				0.25
No	164 (78.4)	45 (21.5)	Ref	
Yes	197 (82.7)	41 (17.2)	0.8 (0.5–1.1)	
Tobacco use				0.21
No	187 (78.5)	51 (21.4)	Ref	
Yes	174(83.2)	35 (16.7)	0.7 (0.5–1.1)	
PPI use				0.04
No	248 (78.2)	69 (21.7)	Ref	
Yes	113 (86.9)	17 (13.0)	0.6 (0.3–0.9)	
GERD				0.96
No	230 (80.7)	55 (19.3)	Ref	
Yes	131 (80.8)	31 (19.1)	0.9 (0.6–1.4)	
Sum of three factors (tobacco use, drinking, and GERD)				0.41
0	169 (77.5)	49 (22.4)	Ref	
1	153 (83.6)	30 (16.3)	0.7 (0.4–1.0)	
2	34 (85.0)	6 (15.0)	0.6 (0.3–1.4)	
3	5 (83.3)	1 (16.6)	0.7 (0.1–4.5)	

(CI 0.3–0.9)]. Age, sex, family income, tobacco use and drinking, and GERD were not associated with the number of MN in the oral mucosal cells of the investigated older participants.

Considering the small number of smokers and drinkers in some categories, they were grouped as follows: tobacco use (YES = low and moderate risks; NO = never used), and drinking (YES = low, moderate, and high risks; NO= never used). In addition, three relevant risk factors (GERD, drinking, and tobacco use) for oral cell DNA mutation were grouped and associated with the presence of MN, showing no statistical significance (Table 1).

The mean MC found for all collected cell samples was 15/1,000 cells and the median was 11/1,000 cells. For the sake of dichotomization, the median was considered the parameter (≤ 11 MCs and > 11 MCs) and 49.2% of the participants showed more than 11 MCs. Table 2 shows that the prevalence ratio of MCs and the respective

Table 2. Descriptive analysis and Poisson regression (PR) with confidence interval (CI) based on the dichotomized variable "metanuclear changes" (MCs) (n = 447 individuals).

Variable	MCs		PR (CI)	p-value
	≤ 11	> 11		
	227 (50.8%)	220 (49.2%)		
Sex				0.08
Male	106 (46.7)	121 (53.3)	Ref	
Female	121 (55.0)	99 (45.0)	0.8 (0.6–1.0)	
Age (in years)				0.92
60–65	77 (52.0)	71 (47.9)	Ref	
66–74	75 (50.6)	73 (49.3)	1.0 (0.8–1.2)	
75 or older	75 (49.6)	76 (50.3)	1.0 (0.8–1.3)	
Family income				0.39
< US$ 500.00	40 (55.5)	32 (44.4)	Ref	
≥ US$ 500.00	187 (49.8)	188 (50.1)	1.1 (0.8–1.4)	
Drinking				0.06
No	116 (55.5)	93 (44.5)	Ref	
Yes	111 (46.6)	127 (53.3)	1.1 (0.9–1.4)	
Tobacco use				0.68
No	123 (51.6)	115 (48.3)	Ref	
Yes	104 (49.7)	105 (50.2)	1.0 (0.8–1.2)	
PPI use				0.83
No	162 (51.1)	155 (48.9)	Ref	
Yes	65 (50.0)	65 (50.0)	1.0 (0.8–1.2)	
GERD				0.73
No	143 (50.1)	142 (49.8)	Ref	
Yes	84 (51.8)	78 (48.1)	0.9 (0.7–1.1)	
Sum of three factors (tobacco use, drinking, and GERD)				0.27
0	107 (49.0)	111 (50.9)	Ref	
1	101 (55.1)	82 (44.8)	0.8 (0.7–1.0)	
2	17 (42.5)	23 (57.5)	1.1 (0.8–1.5)	
3	2 (33.3)	4 (66.6)	1.3 (0.7–2.3)	

confidence intervals did not present statistical significance ($p > 0.05$) when associated with any of the variables studied. Although the absolute values with >11 MCs were almost 10% higher for drinking and 2% for tobacco use (Table 2), no statistical significance was observed in these associations ($p = 0.06$ and $p = 0.68$, respectively).

So, age, sex, family income, tobacco use and drinking, PPI use, or GERD were not associated with the number of MCs in the oral mucosal cells of the investigated older participants (Table 2).

Discussion

The present study, conducted with a population of older people from a rural area, partially rejected the experimental hypothesis because it showed that factors such as sex, deleterious habits, and the presence of GERD did not significantly affect the number of MN and MCs in the oral epithelial cells of this population. However, there was a significant influence of PPI use on MN.

A study[28] compared 80 healthy young individuals (aged 19 to 29 years) with older people (≥ 60 years), associating sex and age with the frequency of MN and MCs. After applying a questionnaire and collecting epithelial cells from the oral mucosa of the participants, the authors concluded that the occurrence of MN was higher among the older people, irrespective of sex. Another study[29] evaluated 245 individuals with a mean age of 60.5 years by collecting mucosal cells from the mid-oesophagus (approximately 30 cm from the upper dental arch) and concluded that the frequency of MN did not show significant differences ($p > 0.05$) regarding sex and place of residence (rural or urban area) of the individuals. Similarly, the present study did not show significant differences in the number of MN and MCs between men and women. The age range of the older individuals also did not affect the number of MN and MCs, suggesting homogeneous exposure of the study population to the genotoxic factors throughout life.

Tobacco use has been described as the main risk factor for the development of malignant and carcinogenic oral lesions, and the combination of deleterious habits such as drinking and tobacco use may increase the prevalence of oral cancer.[30] A greater aggressiveness of oral carcinoma in drinkers and smokers may be due to the increased permeability of the cell membrane caused by ethanol and the consequent exposure of intracellular content to tobacco as a carcinogen.[31] A study[32] evaluated the genotoxic effects of tobacco and noted a significant increase in the frequency of MN in individuals that either smoked or chewed tobacco, or smoked and chewed tobacco concomitantly, when compared to the control group. These studies suggested that drinking and tobacco use are risk factors for the development of precancerous lesions.

The number of MN in normal oral mucosal cells may reach 2 MN/1,000 cells,[33] which is in agreement with the cell analyses of the present study, suggesting absence of genotoxicity in the oral mucosal cells of the evaluated population. The present study also did not find a significant difference for the number of MN and MCs among tobacco users or drinkers or among those who used both substances, although the absolute values in individuals with > 11 MCs were almost 10% greater for drinkers and 2% higher for tobacco users (Table 2). This result may be explained by the fact that tobacco use and drinking were moderate in the evaluated population, probably not reaching the thresholds for the microscopic diagnosis of genotoxicity, which consists of a minimum consumption of 30 cigarettes/day.[33,34]

A study[1] evaluated 120 Brazilian farmers for their exposure to tobacco and pesticides, warning about the importance of biomonitoring studies in populations exposed to genotoxic agents, particularly rural workers. The authors also reported that the MN test is easy to perform, minimally invasive, and inexpensive, and it may be used as a tool for epidemiological and genotoxic investigations in individuals with harmful health habits, thus justifying the use of such methodology in the present study.

PPIs are used for the long-term treatment of gastroesophageal disorders, including acid reflux. However, there are growing concerns about the improper and excessive use of these inhibitors. Yang et al.[35] assessed 21 Wistar rats, simulating the

long-term use of omeprazole. After euthanizing the animals, morphological changes were found in the bile duct, including ductal epithelial proliferation, micropapillary growth of the biliary epithelium, focal narrowing, and bile duct obstruction. These are characteristics of precancerous lesions and indicate a high-fat diet. Although omeprazole has been subjected to a wide range of genotoxicity tests, which were all negative, the ability of this compound to interact with DNA and induce non-programmed DNA synthesis in the gastroesophageal mucosa has been discussed. In this sense, Mereto et al.[36] assessed the gastric mucosa of rats that received 100 mg/kg/day of omeprazole for 14 days, but they did not find a significant increase in the number of MN. Similarly, Sinués et al.[37] performed a clinical trial with 33 healthy volunteers who received 20 mg/day of omeprazole for 14 days. Although the number of MN slightly increased, omeprazole did not present genotoxic effects after 14 days of treatment. In the present study, PPI use was a protective factor for the prevalence of MN ($p = 0.04$), but with no clinical significance, given that the frequency of up to 2 MN/1,000 cells is considered normal.[34-38]

Considering oral cancer, it is essential to look at the moment of screening, as it plays a fundamental role in understanding the prognosis of the disease. Critical signs and symptoms that can be identified during the initial screening can increase a patient's chances of survival. Reports suggest that socioeconomic factors, lack of public awareness, and delays in primary health care centers are some of the main parameters that contribute to patient mortality and morbidity. The conventional visual examination of oral lesions can effectively monitor patient mortality in the presence of risk factors. Yet, some disadvantages limit the clinical use of this method. Thus, screenings that efficiently differentiate benign from malignant lesions, as well as provide information about the early stage of cancer, facilitate the detection of complications associated with the diagnosis of oral cancer.[39]

In addition, previous studies[40,41] have reported that diseases such as oral submucous fibrosis, mucosal ulcerations, and xerostomia are significantly more common in patients with GERD. In a recent study, Gilligan et al.[42] claimed that chronic traumatic oral lesions (caused by acids, for example) may eventually predispose to oral carcinomas. Thus, considering that subclinical cellular alterations, such as the number of micronuclei, can also precede oral carcinomas,[43] the present study performed a population-based screening test, correlating the frequency of MN and MCs with risk factors such as the presence of GERD, smoking, and drinking. In addition, a systematic review[44] indicated that some PPIs might induce genomic instability and increase the risk of certain types of cancer, suggesting caution with long-term therapeutic strategies and self-medication with PPIs.

GERD is a highly prevalent disorder in the global population. According to Mamede et al.,[45] clinical evidence indicates that GERD may cause changes to tongue tissues. For Lipan et al.,[46] the reflux that advances to the laryngopharynx and later to other head and neck regions, including the oral cavity, may result in relevant problems. Kuo et al.[14] analyzed 39,845 GERD patients and found 98 cases of head and neck cancer, in which oropharyngeal and hypopharyngeal cancers in men were statistically associated with GERD.

A study[47] on mild and severe esophagitis and cancer cases with the comet assay and biopsy of the distal third of the esophagus reported damage to the DNA of esophageal mucosal cells. Moreover, the study proved that most DNA changes were directly connected to the level of inflammation in the region. Nevertheless, the relationship between GERD and extraesophageal cancers is controversial.[48] In this sense, the results of the present study indicate that the assessed population did not show a relationship between GERD and increased MN and MCs in the oral mucosal cells, suggesting that the oral mucosa is not deleteriously affected by the presence of GERD in the population studied. Yet, GERD patients have multiple changes in pH and salivary microbiome when compared to healthy patients.[49] A higher prevalence of dental erosion and caries was reported for patients with GERD as compared to healthy individuals, and the pathophysiological mechanisms of GERD involve changes in saliva physiology.[50] As the present study also evaluated deleterious habits (smoking and drinking) directly related to the oral

mucosa, and saliva is present in all regions of the oral cavity, the oral cells were collected from the right and left buccal mucosae, which are easily accessible sites, avoiding nausea and discomfort for the older people and also optimizing cell collection.

Note that the instrument used to assess the presence of GERD was subjective (a validated questionnaire), which is less robust than objective assessments such as endoscopy and pH monitoring, and this may be considered a limitation of this study. In addition, other factors such as the time for the diagnosis of GERD, the length of PPI use, and exposure to toxic products such as fertilizers and pesticides were not considered in the present study. Thus, future studies should focus on different populations, comparing residents of urban and rural areas, young adults and older people, and inhabitants of distinct geographical regions.

Conclusion

Considering the methodology used and the evaluated population (older individuals from a rural area), it can be concluded that factors such as sex, age greater than 60 years, tobacco use and drinking, and the presence of GERD did not affect the number of MN and MCs in the epithelial cells of the oral mucosa.

References

1. Tomiazzi JS, Judai MA, Nai GA, Pereira DR, Antunes PA, Favareto AP. Evaluation of genotoxic effects in Brazilian agricultural workers exposed to pesticides and cigarette smoke using machine-learning algorithms. Environ Sci Pollut Res Int. 2018 Jan;25(2):1259-69. https://doi.org/10.1007/s11356-017-0496-y
2. Instituto Brasileiro de Geografia e Estatística. [Demographic census[. Brasília, DF: Instituto Brasileiro de Geografia e Estatística; 2010.
3. Hochman G. Salt as solution? Health policies and endemic rural diseases in Brazil (1940-1960). Sociologias. 2010 Aug;12(24):158-93. https://doi.org/10.1590/S1517-45222010000200007.
4. Fos P, Hutchison L. The state of rural oral health: a literature review. In: Gamm LD, Hutchison LL, Dabney BJ, Dorsey AM, editors. Rural healthy people 2010: a companion document to healthy people 2010. College Station: The Texas A&M University System Health Science Center, School of Rural Public Health, Southwest Rural Health Research Center; 2003. V. 2, p. 131-44.
5. Instituto Brasileiro de Geografia e Estatística. Síntese de indicadores sociais: uma análise das condições de vida da população brasileira. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2013 [cited 2022 Apr 20]. Available from: https://biblioteca.ibge.gov.br/?id=266778&view=detalhes
6. Alcântara AO, Duarte AG, Frota MH. Old age and rural space: (re)designs of speeches. Rev Kairós Gerontol. 2015;18(2):209-26. https://doi.org/10.23925/2176-901X.2015v18i2p209-226
7. Tavares DM, Gomes NC, Dias FA, Santos NM. Factors associated with the quality of life of elderly people with osteoporosis living in rural areas. Esc Anna Nery Rev Enferm. 2012;16(2):371-8. Htttps://doi.org/10.1590/S1414-81452012000200023
8. Travassos C, Viacava F. [Access to and use of health services by elderly residents in rural areas, Brazil, 1998 and 2003]. Cad Saúde Pública. 2007;23(10):2490-502. Portuguese. https://doi.org/10.1590/S0102-311X2007001000023
9. Tavares DM, Fachinelli AM, Dias FA, Bolina AF, Paiva MP. [Predictors of the quality of life of urban and rural elderly people]. Rev Baiana Enferm. 2015;29(4):361-71. Portuguese. https://doi.org/10.18471/rbe.v29i4.13359
10. Oliveira JM, Pinto LO, Lima NG<, Almeida GC. [Oral cancer: assessment of academic dentistry and nursing knowledge as for the risk factors and diagnostic procedures]. Rev Bras Cancerol. 2013 abr/jun;59(2):211-8. Portuguese. https://doi.org/10.32635/2176-9745.RBC.2013v59n2.526
11. Hyman JJ, Reid BC. Epidemiologic risk factors for periodontal attachment loss among adults in the United States. J Clin Periodontol. 2003 Mar;30(3):230-7. https://doi.org/10.1034/j.1600-051X.2003.00157.x
12. Moynihan P. The interrelationship between diet and oral health. Proc Nutr Soc. 2005 Nov;64(4):571-80. https://doi.org/10.1079/PNS2005431
13. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol. 2003 Dec;31 Suppl 1:3-23. https://doi.org/10.1046/j..2003.com122.x
14. Kuo CL, Chen YT, Shiao AS, Lien CF, Wang SJ. Acid reflux and head and neck cancer risk: A nationwide registry over 13 years. Auris Nasus Larynx. 2015 Oct;42(5):401-5. https://doi.org/10.1016/j.anl.2015.03.008

15. UICC. International Union Against Cancer: Introduction UICC global cancer control. Geneve: UICC; 2005.
16. Centers for Disease Control and Prevention. The National Institute for Occupational Safety and Health (NIOSHI). Exposome and exposomics. 2021 [cited 2021 apr 2]. Available from: https://www.cdc.gov/niosh/topics/exposome/default.html
17. Cavalcante DN, Crispim BD, Grisolia BB, Viana LF, Maran NH, Solórzano JC, et al. Effects of age, sex, medication, and environmental conditions on genetic alterations in oral mucosa cells. Heliyon. 2019 Jun;5(6):e01840. https://doi.org/10.1016/j.heliyon.2019.e01840
18. Jois HS, Kale AD, Mohan Kumar KP. Micronucleus as potential biomarker of oral carcinogenesis. Indian J Dental Adv. 2010 Apr/Jun;2(2):1-5.
19. Freita VS, Lopes MA, Meireles JR, Reis L, Cerqueira EM. Genotoxic effects of factors considered of risk for the buccal cancer. RBSP. 2014;29(2):189-99. https://doi.org/10.22278/2318-2660.2005.v29.n2.a1001
20. Shashikala R, Indira AP, Manjunath GS, Rao KA, Akshatha BK. Role of micronucleus in oral exfoliative cytology. J Pharm Bioallied Sci. 2015 Aug;7(6 Suppl 2):S409-13. https://doi.org/10.4103/0975-7406.163472
21. Malta M, Cardoso LO, Bastos FI, Magnanini MM, Silva CM. STROBE initiative: guidelines on reporting observational studies. Rev Saúde Pública. 2010 Jun;44(3):559-65. https://doi.org/10.1590/S0034-89102010000300021
22. WHO ASSIST Working Group. The alcohol, smoking and substance involvement screening test (ASSIST): development, reliability and feasibility. Addiction. 2002 Sep;97(9):1183-94. https://doi.org/10.1046/j.1360-0443.2002.00185.x
23. Fornari F, Gruber AC, Lopes AB, Cecchetti D, Barros SG. [Symptom's questionnaire for gastroesophageal reflux disease]. Arq Gastroenterol. 2004;41(4):263-7. Portuguese. https://doi.org/10.1590/S0004-28032004000400012
24. Silva RR, De Carli JP, Collares K, Vanini J, Presotto JS, Vargas JE, et al. Influence of smoking on oral cells genotoxicity after at-home bleaching using 22% carbamide peroxide: a cohort study. Clin Oral Investig. 2022 Feb;26(2):1409-16. https://doi.org/10.1007/s00784-021-04115-z
25. Thomas P, Fenech M. Buccal micronucleus cytome assay. In: Didenko VV, ed. DNA damage detection in situ ex vivo and in vivo: methods and protocols. [Place unknown; 2011. (MIMV, v. 682). p. 235-48.
26. Bolognesi C, Knasmueller S, Nersesyan A, Thomas P, Fenech M. 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):100-13. https://doi.org/10.1016/j.mrrev.2013.07.002
27. Bonacina LV, Vargas CD, Vanini J, Zandonà J, Silva RR, Busin CS, et al. Genotoxicity of 22% carbamide peroxide bleaching agent on oral cells using the micronucleus technique. J Clin Diagn Res. 2020;14:ZC14-7. https://doi.org/10.7860/JCDR/2020/43341.13569
28. Kausar A, Giri S, Mazumdar M, Giri A, Roy P, Dhar P. Micronucleus and other nuclear abnormalities among betel quid chewers with or without sadagura, a unique smokeless tobacco preparation, in a population from North-East India. Mutat Res. 2009;677(1-2):72-5. https://doi.org/10.1016/j.mrgentox.2009.05.007
29. Dietz J, Diehl AS, Prolla JC, Furtado CD, Furtado AD. Micronuclei research the esophageal mucosa and its relationship with risk factors with cancer of the esophagus. Rev Assoc Med Bras (1992). 2000;46(3):207-211. https://doi.org/10.1590/s0104-42302000000300004
30. Chaturvedi P, Singh A, Chien CY, Warnakulasuriya S. Tobacco related oral cancer. BMJ. 2019 Jun;365:l2142. https://doi.org/10.1136/bmj.l2142
31. Bezerra NV, Leite KL, Medeiros MM, Martins ML, Cardoso AM, Alves PM, 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 May;23(3):e295-301. https://doi.org/10.4317/medoral.22318
32. Upadhyay M, Verma P, Sabharwal R, Subudhi SK, Jatol-Tekade S, Naphade V, et al. Micronuclei in exfoliated cells: a biomarker of genotoxicity in tobacco users. Niger J Surg. 2019;25(1):52-9. https://doi.org/10.4103/njs.NJS_10_18
33. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, et al. The HUman MicroNucleus project on eXfoLiated buccal cells (HUMN(XL)): the role of life-style, host factors, occupational exposures, health status, and assay protocol. Mutat Res. 2011;728(3):88-97. https://doi.org/10.1016/j.mrrev.2011.06.005
34. Fenech M, Holland N, Zeiger E, Chang WP, Burgaz S, Thomas P, et al. The HUMN and HUMNxL international collaboration projects on human micronucleus assays in lymphocytes and buccal cells: past, present and future. Mutagenesis. 2011 Jan;26(1):239-45. https://doi.org/10.1093/mutage/geq051
35. Yang YS, Chang HW, Lin IH, Chien LN, Wu MJ, Liu YR, et al. Long-term proton pump inhibitor administration caused physiological and microbiota changes in rats. Sci Rep. 2020 Jan;10(1):866. https://doi.org/10.1038/s41598-020-57612-8
36. Mereto E, Ghia M, Martelli A, Brambilla G. Lack of evidence of omeprazole genotoxicity in Sprague-Dawley rats. Mutagenesis. 1993 Sep;8(5):379-86. https://doi.org/10.1093/mutage/8.5.379
37. Sinués B, Fanlo A, Bernal ML, Val M, Mayayo E. Omeprazole treatment: genotoxicity biomarkers, and potential to induce CYP1A2 activity in humans. Hum Exp Toxicol. 2004 Mar;23(3):107-13. https://doi.org/10.1191/0960327104ht431oa
38. Rezende M, De Geus JL, Loguercio AD, Reis A, Kossatz D. Clinical evaluation of genotoxicity of in-office bleaching. Oper Dent. 2016;41(6):578-86. https://doi.org/10.2341/15-207-C

39. Chakraborty D, Natarajan C, Mukherjee A. Advances in oral cancer detection. Adv Clin Chem. 2019;91:181-200. https://doi.org/10.1016/bs.acc.2019.03.006
40. Warsi I, Ahmed J, Younus A, Rasheed A, Akhtar TS, Ain QU, et al. Risk factors associated with oral manifestations and oral health impact of gastro-oesophageal reflux disease: a multicentre, cross-sectional study in Pakistan. BMJ Open. 2019 Mar;9(3):e021458. https://doi.org/10.1136/bmjopen-2017-021458
41. Shu L, Tong X. Exploring the causal relationship between gastroesophageal reflux and oral lesions: A mendelian randomization study. Front Genet. 2022 Nov;13:1046989. https://doi.org/10.3389/fgene.2022.1046989
42. Gilligan G, Piemonte E, Lazos J, Simancas MC, Panico R, Warnakulasuriya S. Oral squamous cell carcinoma arising from chronic traumatic ulcers. Clin Oral Investig. 2022 Sep;27(1):193-201. https://doi.org/10.1007/s00784-022-04710-8
43. Singam PK, Majumdar S, Uppala D, Kotina S, Namana M, Ayyagari KR. 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. https://doi.org/10.4103/jomfp.JOMFP_221_19
44. Paz MF, Alencar MV, Lima RM, Sobral AL, Nascimento GT, Reis CA, et al. Pharmacological effects and toxicogenetic impacts of omeprazole: genomic instability and cancer. Oxid Med Cell Longev. 2020 Mar;2020:3457890. https://doi.org/10.1155/2020/3457890
45. Mamede RC, Mello-Filho FV, Vigário LC, Dantas RO. Effect of gastroesophageal reflux on hypertrophy of the base of the tongue. Otolaryngol Head Neck Surg. 2000 Apr;122(4):607-10. https://doi.org/10.1067/mhn.2000.98362
46. Lipan MJ, Reidenberg JS, Laitman JT. Anatomy of reflux: a growing health problem affecting structures of the head and neck. Anat Rec B New Anat. 2006 Nov;289(6):261-70. https://doi.org/10.1002/ar.b.20120
47. Bertolozzo EL, Freire-Maia DV, Lerco MM, Plácido MS, Henry MA. Evaluation of DNA damage in the esophageal mucosa and peripheral blood of patients with gastroesophageal reflux disease. Arq Bras Cir Dig. 2010;23:217-21. https://doi.org/10.1590/S0102-67202010000400002
48. Ofman JJ. The relation between gastroesophageal reflux disease and esophageal and head and neck cancers: a critical appraisal of epidemiologic literature. Am J Med. 2001 Dec;111(8 Suppl 8A):124S-9S. https://doi.org/10.1016/S0002-9343(01)00857-9
49. Kawar N, Park SG, Schwartz JL, Callahan N, Obrez A, Yang B, et al. Salivary microbiome with gastroesophageal reflux disease and treatment. Sci Rep. 2021 Jan;11(1):188. https://doi.org/10.1038/s41598-020-80170-y
50. Lechien JR, Chiesa-Estomba CM, Calvo Henriquez C, Mouawad F, Ristagno C, Barillari MR, et al. Laryngopharyngeal reflux, gastroesophageal reflux and dental disorders: A systematic review. PLoS One. 2020 Aug;15(8):e0237581. https://doi.org/10.1371/journal.pone.0237581