RESEARCH ARTICLE

Editorial Process: Submission:07/29/2024 Acceptance:12/15/2024

Effect of Age, Hot Beverages and Tobacco Related Products on Buccal Epithelial Cells of Cigarette Smokers and non-Smokers in Ajman, UAE

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Abstract

Objective: This study aimed to find out the effect of age, hot beverages and tobacco related products on buccal mucosa cells between cigarette smokers and non-smokers in Ajman, UAE. **Methods:** A total of 122 samples were collected, with demographic data including age, hot beverage consumption, cigarette smoking and other tobacco practice using pre-designed questionnaires. Buccal cells were collected, stained, and screened for micronuclei (MN) under a microscope and two evaluators independently assessed all the slides. **Results:** Among the 122 participants, 61.5% were aged ≤35 years, and 38.5% were aged >35 years. All non-smokers had MN values <10, while 87% of smokers had MN values >10 ($p<0.001$), with a trend of dose-dependent relationship between cigarette consumption and MN frequency. Similar patterns were observed in individuals using other forms of tobacco, with 97.4% exhibiting MN values >10 ($p<0.001$). Hot beverage consumption ≥7 cups/day was associated with 87% of subjects having MN values >10, highlighting the pattern of alternative forms of tobacco and high consumption of hot beverages association with elevated MN occurrence. Significant associations were found between MN and variables, except for age. **Conclusion:** The findings underscore the significance of tobacco and hot beverage consumption in MN occurrence, emphasizing the need to address these behaviors to mitigate genotoxicity and associated health risks. Despite age showing no significant correlation with MN frequency within the studied age range, aging combined with cigarette smoking amplifies genetic damage.

Keywords: Micronuclei- smoking- Tobacco- hot beverage- oral cancer

Asian Pac J Cancer Prev, **25 (12)**, 4293-4298

Introduction

The persistent global health concern surrounding smoking and tobacco consumption remains an issue of utmost importance, due to significant morbidity and mortality it inflicts [1]. Projections indicate that by the year 2030, the annual toll of tobacco-related deaths could surge to a staggering 8 million, emphasizing the dire need for comprehensive interventions [2].

In the United Arab Emirates (UAE) tobacco is commonly consumed in the form of cigarettes, medwakh, pipes and shisha [3]. Of particular concern is the well-documented adverse impact of cigarette smoking on oral health, with smoking emerging as a significant risk factor for oral cancer and other disorders [4-7]. The pernicious nature of cigarette smoke, harbouring an array of carcinogens such as nicotine, N-nitrosamines, benzene, and aromatic amines, warrants attention for its potential to induce genotoxicity and inflict DNA damage [8, 9], that can be detected using biomarkers.

Biomarkers can be effectively employed to distinguish various preneoplastic conditions well before clinical symptoms manifest, particularly in high-risk populations [10, 11]. Early biomarkers are typically categorized into three groups: the first group aims to establish exposure to carcinogenic agents, the second group seeks to demonstrate the biological effects on target tissues, and the third group focuses on identifying individual susceptibility [12]. The assessment of micronuclei (MN) scoring using micronucleus assay is recognized as a valuable biomarker for DNA damage, and it holds promise as a prospective predictor of heightened genotoxicity [13].

It is noteworthy that a heightened frequency of micronuclei in the buccal mucosal epithelium has been consistently observed in cigarette smokers, significantly elevating their susceptibility to oral cancer [14]. It is also crucial to emphasize that the assessment of MN in exfoliated oral buccal epithelial cells not only exhibits potential as a reliable diagnostic method but is also nonintrusive, thereby reducing patient discomfort and the invasiveness of the procedure. In addition, the effect of secondary factors like age, usage of hot beverages

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and other tobacco factors also are reported to affect the buccal epithelial cells. Considering all these points, the present study was designed to compare the frequency of micronucleus in buccal mucosa cells of smoker's indicative of chromosomal alterations posing the risk of oral cancer.

Materials and Methods

The ethical approval was obtained by the Institutional Research Board of Gulf Medical University (Ref. No. IRB-COM-STD-41-JUN-2020). Subjects were informed of the objectives of the work, and written consent to participate in this study was obtained. A total of 122 samples were collected (n=90) from the two-study population (62 smokers and 60 non- smokers) between the age group of 25-45 years. The subjects who smoked >10 cigarettes/day at least for 1 year were included in smokers' group. The demographic details (age, genetic disorders, years of smoking, number of X-ray diagnoses, vaccinations, dental problem, medication, smoking, alcohol, etc.) of the participants were taken on a pre-designed questionnaire filled by interviewing all the participants. The questionnaire also included queries which recorded their smoking habits mainly the number of cigarettes smoked/day, years of smoking, use of other forms of tobacco consumption including medwak, shisha (commonly used in middle-east). Under hot beverages (served above 54◦C), the section included queries related to the type of hot beverage (tea/coffee), number of times / days was recorded for the study.

Individuals were asked to rinse their mouth with water. Wooden tongue depressor was used to collect cells from the inner wall of the cheek. The slides were prepared by direct smearing of buccal cells on a clean microscope slide. The smears were air dried, and slides stained for 10 minutes in 2% Giemsa solution followed by air drying after rinsing the stained slide with distilled water. Stained slides were screened for the presence of micronuclei. Exfoliated buccal cells were analyzed under a total magnification of x1000 using a light microscope. Only cells that were not clumped or overlapped and that contained intact nuclei were included for the analysis of MN. The frequency of MN and degenerative nuclear alterations (nuclear buds, pyknotic, karyolytic and karyorrhectic cells) in differentiated human buccal cells were recorded.

Two evaluators individually assessed all the slides. Each evaluator provided a count for each of the duplicate slides, and the average values were computed to account for both experimental and scorer differences.

Statistical Analysis

The statistical analysis was done in SPSS-28. The Chi-Square test was used to assess the association and simple and multiple logistic regression was used to analyze the data, to calculate the crude and adjusted OR. The $p \leq 0.05$ is considered as significant

Results

The demographic details of the participants are summarized in Table 1. Regarding age distribution, 75 participants (61.5%) were 35 years old or younger, while 47 participants (38.5%) were over the age of 35. It also includes details about smoking habits and consumption of hot beverages.

MN counts were recorded for all participants, as detailed in Table 2. Among non-smokers, all participants had fewer than 10 MN. In contrast, 87% of smokers exhibited MN counts exceeding 10. The data also demonstrated a clear trend: as the number of cigarettes smoked increased, the incidence of MN counts greater than 10 rose accordingly. Out of 15 participants consuming seven or more cups of hot beverages per day, 13 (87%) had MN counts exceeding 10, while in 42 participants consuming hot beverages four to six times daily, 22 had MN counts exceeding 10.

The crude and adjusted odds ratio for MN using logistic regression has been calculated and depicted in Table 3. For smoking, the crude OR observed was 202, which showed the likelihood of more than 10 MN is 202 times higher among smokers compared to those who are not smoking.

For other tobacco use, the crude OR observed was 130.4, which showed the likelihood of more than 10 MN is 130.4 times higher among using other types of tobacco products compared to those who are not using.

Table 1. Number of Participants Consuming Hot Beverage, Cigarettes, and Other Tobacco Products

Variables	Group		No. of participants	%
Age	<=35		75	61.5
	>35		47	38.5
Hot beverage consumers (no. of cups/day)	<=3		65	53.3
	6-Apr		42	34.4
	>=7		15	12.3
No. of Cigarette smoke/day	Non-smokers		60	49.2
	Smokers (n-62)	<=10	19	15.6
		10-15	29	23.8
		>15	14	11.5
Other tobacco use	No		84	68.9
	Yes		38	31.1

Table 2. Number of Micronuclei Compared with Variable Groups.

Variable	Group	Total no. of participants	No. of. Participants with <=10 MN (%)	No. of. Participants with >10 MN (%)		P
Age (years)	<=35	75	38(50.7)	37 (49.3)		NS
	>35	47	30 (63.8)	17 (36.2)		
Smoking	Non-smoker	60	60 (100.0)	--	--	<0.001
	Smoker	62	8 (12.9)	54 (87.1)		
No. of cigarette	Non-users	60	60 (100.0)	--	--	<0.001
	<=10	19	4 (21.1)	15 (78.9)		
	10-15	29	2 (6.9)	27 (93.1)		
	>15	14	1 (7.2)	13 (92.8)		
Other tobacco	No	84	67 (79.8)	17 (20.2)		<0.001
	Yes	38	1 (2.6)	37 (97.4)		
Hot beverage (no. of cups / day)	<=3	65	46 (70.8)	19 (29.2)		<0.001
	4-6	42	20 (47.6)	22 (52.4)		
	>=7	15	2 (13.3)	13 (86.7)		

*p<0.05

Table 3. Crude and adjusted Odds Ratio for Micro Nuclei Using Logistic Regression

Variable	Group	COR	CI	AOR	CI
Smoking	No	1	--	1	--
	Yes	202.5	41.2 – 995.5	227.1	38.0 – 1355.9
Hot beverages	<=3	1	--	1	--
	4-6	2.7	1.2 – 5.9	4.7	0.9 – 24.8
	>=7	14.95	3.1 – 72.5	3.6	0.4 – 30.1
Other tobacco	No	1	--	1	--
	Yes	130.4	16.8 – 1014.1	--	--

For hot beverages consumption, the crude OR observed was 2.7, which showed the likelihood of more than 10 MN is 2.7 times higher among those who are using 4-6 cups/day of any hot beverages compared to those who are using less than or equal to 3 cups/day. Moreover, those who are taking more than or equal to 7 cups per day is 15 times high risk compared to those who are taking <=3 cups per day.

Discussion

Tobacco use is a significant public health concern worldwide, and its detrimental effects are well-documented [2, 15]. The present study aimed to conduct a comparative analysis of MN frequency in buccal epithelial cells of cigarette smokers and non-smokers in Ajman, UAE. The presence of MN in the buccal cells serves as an important biomarker for assessing genotoxicity and the potential risk of developing oral cancer associated with tobacco exposure [16]. Moreover, the MN assay has demonstrated a sensitivity of 94%, specificity of 100%, and an accuracy of 95%, establishing it as a reliable predictor [17].

The findings of this study are consistent with a growing body of evidence that highlights the genotoxic effects of cigarette smoking. The induction of nicotine-related DNA damage is believed to be a consequence of oxidative stress [18-20]. MN can arise from either acentric chromosome fragments or whole chromosomes that lag during anaphase and subsequently remain outside the daughter nuclei [21]. These micronuclei can originate from chromosome breakage resulting from either unrepaired or misrepaired DNA lesions or chromosome malsegregation due to mitotic malfunctioning [14, 22-23].

Numerous studies in the past have documented an elevated occurrence of MN in the buccal mucosal epithelium of individuals who smoke cigarettes [16, 24-25]. Our findings closely parallel those of Balraj et al. [25], consolidating the link between cigarette smoking and MN formation. For instance, in our study, participants consuming up to 10 cigarettes exhibited 21.1% with MN counts of 10 or fewer, echoing similar trends observed by Wagh et al. [26] and Motgi et al. [27], who found lower MN counts in individuals with lower cigarette consumption. Likewise, individuals smoking between 10 - 15 cigarettes demonstrated a considerable majority (93.1%) with MN counts exceeding 10, consistent with the findings of Upadhyay et al. [24] and Balraj et al. [25] who reported elevated MN levels in this population. Furthermore, our research identified that participants smoking more than 15 cigarettes displayed 14.3% with MN counts of 10 or fewer and 85.7% more than 10 MN, corroborating the observations of Balraj et al. [25] and Mohammed et al. [13] regarding the heightened MN counts in chronic smokers, particularly those with prolonged smoking durations.

Therefore, the convergence of our results with multiple studies across different populations provides robust evidence supporting the association between cigarette smoking intensity and MN formation.

The higher prevalence of MN in the buccal epithelial cells of smokers compared to non-smokers indicates the presence of chromosomal alterations. This is a concerning observation, as it is well-established that chromosomal instability is a hallmark of cancer development [28-30]. Cigarette smoke is known to contain numerous carcinogens, many of which have been identified as activators of DNA adducts, causing DNA damage and chromosomal alterations [31]. The diverse range of chemical compounds in cigarette smoke, estimated at 7357, with 70 confirmed carcinogens (such as nicotine, N-nitrosamines, benzene, and acetaldehyde), play a role in the observed genotoxicity and contribute to the formation of MN [32]. These MN arise from mitotic errors or DNA damage, underscoring the complex and multifaceted genotoxicity of tobacco [33].

The dose-dependent effect of cigarette smoking on the frequency of MN is another noteworthy finding in this study. As the number of cigarettes smoked per day increased, the likelihood of having more than 10 MN also increased. This is in line with previous research indicating that the genotoxic effects of tobacco are proportional to the duration and intensity of exposure [34]. The fact that this relationship is consistent with the findings of this study emphasizes the importance of reducing tobacco consumption to mitigate genotoxicity and the associated cancer risk.

Furthermore, the use of alternative forms of tobacco was significantly associated with an increased frequency of MN. These findings highlight that various tobacco products, not just cigarettes, contribute to genotoxicity, and the prevalence of MN serves as a sensitive marker for assessing the health risks associated with these products [9].

An interesting study done by Ernst et al, [35] has reported that the primary reason of upper digestive tract cancer in drinkers of very hot beverages is not the genetic damage, but an upsurge of the cell division rate due to cytotoxic effects which take place at temperatures above 60 °C . In our study also, hot beverage consumption was found to be associated with an elevated MN frequency, which may be attributed to the potential interaction between hot beverages and tobacco exposure, as both factors have been independently associated with an increased risk of oral cancer [36-37]. By comparing these two observations, it may be concluded that hot beverage consumers along with smoking or any other tobacco consumption are highest risk of cytotoxic effects and hence the risk of cancer. These risk factors emphasize the importance of public health efforts to educate the population about the dangers of tobacco and its potential interactions with other lifestyle factors.

Notably, the age of the participants did not show a significant association with MN frequency. This suggests that the genotoxic effects of tobacco exposure are consistent within the age range studied (25-45 years), emphasizing the harmful impact of tobacco across different age groups. However, the findings of Hilada Nefic et al. [38] provided further insight on the association between ageing, tobacco use, and genetic harm. They show that ageing increases the quantity of micronuclei and other biomarkers of DNA damage. Cigarette smoking aggravates this effect, resulting in increased frequency of MN and numerous types of damaged cells, including nuclear buds, pyknotic, karyolytic, and karyorrhectic cells.

When both sets of observations are taken into account, it is clear that, while age does not directly correspond with MN frequency within a specific age range, ageing does contribute to genetic damage, and tobacco smoking considerably amplifies this effect. This emphasises the significance of addressing tobacco use, regardless of age, to reduce its negative influence on genetic integrity and general health.

In conclusion, this study provides further evidence of the direct genotoxic effects of tobacco and hot beverages on buccal epithelial cells. The presence of MN in these cells serves as a valuable biomarker for assessing the risk of oral cancer. These findings reinforce the importance of early detection and preventive measures for individuals at risk due to tobacco exposure. Public health initiatives, including smoking cessation programs and awareness campaigns, should be promoted to reduce the incidence of oral cancer and its associated morbidity and mortality in the UAE and globally. Additional research is needed to delve deeper into the mechanisms of tobacco-induced genotoxicity and to identify potential interventions to reduce these risks further.

Author Contribution Statement

PS- conceptualization, methodology, investigation, analysis and writing–original draft. RK-Data curation, Formal analysis, and writing–review and editing. NS-Investigation, visualization, and writing–review and editing. JS- Statistical Analysis. All authors contributed to the article and approved the submitted version.

Acknowledgements

We thank Dr Kalpana Golani for providing support in histopathological study.

Approval

This study was approved by the Institutional Research Board, Gulf Medical University, Ajman, UAE (Ref. No. IRB-COM-STD-41-JUN-2020).

Data Availability

The datasets presented in this study is available in Thumbay labs, GMU Ajman, UAE and the investigators in the university repositories. The raw data can be made available whenever required, without undue reservation

Ethical Declaration

Approval This study was approved by the Institutional Research Board, Gulf Medical University, Ajman, UAE (Ref. No. IRB-COM-STD-41-JUN-2020). The study was conducted in accordance with the local legislation

and institutional requirements. The participants provided their written informed consent to participate in this study.

Conflict of Interest

The authors declare that they have no conflict of interest

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