Detection of Micronuclei in Oral Mucosa among Saudi Smokers Using Papanicolaou Stain

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Abstract: *Background: The presence of micronuclei in oral epithelial cells is considered a marker of genotoxicity, which can be identified using exfoliative cytology. Objective: To investigate the presence of micronuclei in the oral mucosa of Saudi smokers using the Papanicolaou stain technique, and to compare these findings with non - smokers. Materials and methods: We conducted a cross - sectional study in KSA from May to October 2023. We collected cytologic oral smear samples from 200 volunteers, of which 100 were smokers and the remaining 100 were non - smokers. We then stained them using the Papanicolaou staining procedure to detect the presence of micronuclei. Results: The mean micronuclei values per 1000 cells were 3.8 ± 1.09 for smokers and 1.85 ± 1.04 for nonsmokers. Smokers had significantly higher values of micronucleated cells compared to nonsmokers, Conclusion: We concluded that the micronuclei count can be used as an early indicator for alterations of oral mucosa and exfoliative cytology represents an accessible tool which could be applied for mass screening.*

Keywords: Micronuclei, Oral Mucosa, Papanicolaou Stain

1. Introduction

Cigarette smoking is a significant public health concern globally, with adverse effects on various organ systems, including the oral cavity. In Saudi Arabia, as in many other countries, smoking remains prevalent despite extensive awareness campaigns and regulatory measures. According to the World Health Organization (WHO), approximately 17.8% of adults in Saudi Arabia were smokers as of 2019 [1]. This high prevalence underscores the urgent need to investigate the potential health consequences of smoking in this population.

One of the well - established consequences of smoking is its genotoxic effects, which can lead to DNA damage and chromosomal instability. Micronuclei, small extranuclear bodies containing chromosomal fragments or whole chromosomes, are widely recognized as biomarkers of genotoxicity [2]. The presence of micronuclei in exfoliated epithelial cells of the oral mucosa reflects ongoing cellular damage and has been associated with various factors, including exposure to carcinogens such as those present in tobacco smoke [3].

The Papanicolaou stain, commonly known as the Pap stain, is a widely used cytological technique for staining cells in clinical practice, particularly in cervical cancer screening. The Pap stain has also been utilized for the detection of micronuclei in oral mucosal cells, offering a simple, cost - effective, and reproducible method for assessing genotoxicity [4].

Despite the wealth of evidence linking smoking to genotoxic effects and the utility of the Pap stain in micronuclei detection, there remains a paucity of research specifically investigating micronuclei in the oral mucosa of Saudi smokers using this technique. Therefore, this study aims to address this gap by evaluating the presence and frequency of micronuclei in the oral mucosa of Saudi smokers using the Pap stain. By comparing these findings with non - smokers, we seek to provide insights into the genotoxic effects of tobacco smoke exposure on oral epithelial cells in the Saudi population, thereby contributing to our understanding of tobacco - related health risks in this region.

2. Materials and Methods

2.1 Study Design and Participants

This cross - sectional study was conducted in the Kingdom of Saudi Arabia (KSA) from May to October 2023. A total of 200 volunteers were recruited for the study, comprising 100 smokers and 100 non - smokers., through convenience sampling. Informed consent was obtained from all participants prior to their inclusion in the study.

2.2 Sample Collection

A wooden tongue depressor was utilized to gather exfoliative cells from the oral mucosa, including the dorsal surface of the tongue and both cheeks. The cells were subsequently applied directly onto a pristine glass slide and promptly immobilized in 95% ethyl alcohol while still damp.

Specimens of buccal smears were forwarded to the histopathology laboratory at Rayyan College of Medicine in Saudi Arabia for the purpose of staining and subsequent diagnosis.

Volume 13 Issue 4, April 2024
Fully Refereed | Open Access | Double Blind Peer Reviewed Journal
www.ijsr.net

Paper ID: SR24412195110   DOI: https://dx.doi.org/10.21275/SR24412195110   1058

Papanicolaou Stain

After being fixed in ethanol, the smears were next hydrated for a duration of two minutes in a sequence of ethanol solutions, with decreasing concentrations ranging from 95% to 70%, and finally in distilled water. The smears were stained with Harris hematoxylin for a duration of five minutes to make the nuclei visible. They were then rinsed in distilled water to eliminate any excess stain. Next, the smears were differentiated in a solution of 0.5% aqueous hydrochloric acid for a period of 10 seconds to remove any remaining stain particles. Finally, the smears were rinsed in distilled water to stop the decolorization process. The smears were subsequently dehydrated in a series of ethanol solutions, starting from 70% and gradually increasing to a concentration twice that of 95%, with each step lasting two minutes. Prior to dehydration, the smears were stained blue in alkaline water for a duration of four seconds. After being incubated for two minutes in Papanicolaou Orange G6 solution, the smears were rinsed with 95% ethanol and then incubated for three minutes in Papanicolaou EA50 staining solution. The purpose of this process was to evaluate the smears for cytoplasmic staining. The smears were subsequently desiccated in 95% pure ethanol, rinsed with xylene, and affixed onto Dibutyl phthalate Polystyrene Xylene (DPX) (5).

Micronuclei Analysis:

The stained slides were examined under a light microscope by two independent observers who were blinded to the participants' smoking status. Micronuclei were identified based on established criteria, including their round or oval shape, smooth contour, similar staining intensity to the main nucleus, and presence within or adjacent to the main nucleus. The number of micronuclei was counted per 1000 epithelial cells, and the mean micronuclei frequency was calculated for each participant.

Statistical Analysis:

Data analysis was conducted using IBM SPSS Statistics version 20 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean, standard deviation, median, and range, were calculated for demographic variables such as age and gender among smokers and non - smokers. A p - value less than 0.05 was considered statistically significant, indicating a significant difference in micronuclei frequency between smokers and non - smokers.

Ethical Considerations:

All participants provided written informed consent before enrollment, and confidentiality of their personal information was strictly maintained throughout the study.

3. Results

The table 1 shows that the proportion of smokers tends to decrease with age, with the highest percentage observed in the 41 - 50 age group (24%) and the lowest in the >60 age group (21%). Conversely, nonsmokers show an increasing trend with age, with the highest percentage in the 41 - 50 age group (29%) and the lowest in the >60 age group (10%). p. value (P> 0.52).

Among smokers, 75% were male and 25% were female, with a mean age of 50.36 years (±1.41 SD). In contrast, among nonsmokers, 53% were male and 38% were female, with a slightly lower mean age of 49.52 years (±1.65 SD) as shown in table 2.

The mean values of the mononuclear cell (MNC) count in the study groups. Among smokers, 13% of the samples contained MNC, with a mean MNC count of 2.48 ± 0.91. In contrast, only 2% of samples from nonsmokers contained MNC, with a mean MNC count of 2.39 ± 1.07. The p - value associated with the comparison between smokers and nonsmokers is 0.042, indicating a statistically significant difference in MNC count between the two groups as shown in table 3.

Among individuals aged 18 - 30, no samples containing MNC were reported, irrespective of smoking status. In the 31 - 40 age group, one sample containing MNC was observed among smokers, while none were reported among non - smokers. MNC counts increased with age, particularly in the 51 - 60 age group, where eight samples were observed among smokers and one among non - smokers. A similar trend was observed in individuals over 60 years old, with two samples containing MNC among smokers and one among non - smokers. Overall, smokers tended to have higher MNC counts compared to non - smokers within each age group as shown in figure 1

Table 1: Distribution of the study population by age

Age (Year)	Smoker (n=100)		Nonsmoker (n=100)		P - value
	N	%	N	%	
18 - 30	21	21	25	25	
31 - 40	20	20	21	21	
41 - 50	24	24	29	29	0.52
51 - 60	14	14	15	15	
>60	21	21	10	10	

Table 2: Demographic characteristics of the study groups

Study group	Male	Female	Age Mean ±SD
Smoker	75 (75%)	25 (25%)	50.36 ± 1.41
Nonsmoker	62 (53%)	38 (38%)	49.52 ± 1.65

Table 3: Mean values of the MNC count in the study groups

Study group	Number of samples containing MNC	MNC Count Mean ± SD	P Value
Smoker	13 (13%)	2.48 ± 0.91	0.042
Nonsmoker	2 (2%)	2.39 ± 1.07	

Volume 13 Issue 4, April 2024
Fully Refereed | Open Access | Double Blind Peer Reviewed Journal
www.ijsr.net

Paper ID: SR24412195110   DOI: https://dx.doi.org/10.21275/SR24412195110   1059
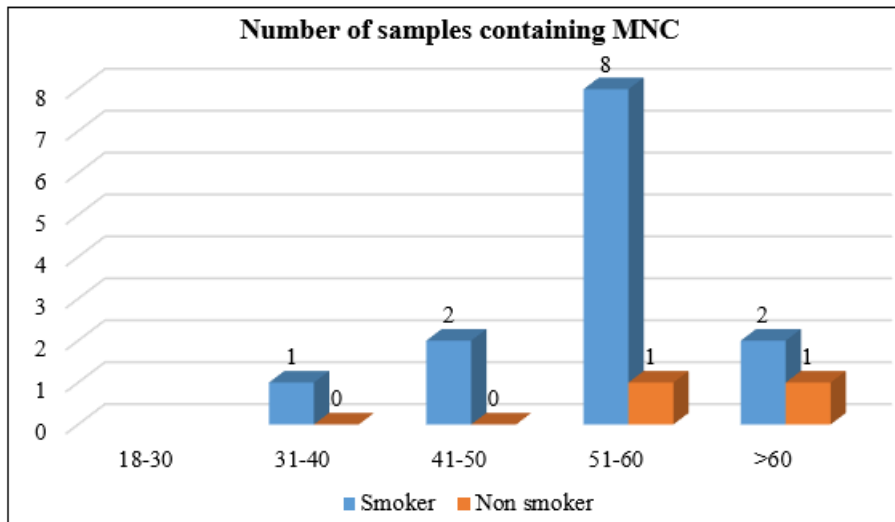
Figure 1: The number of samples containing MNC categorized by age group and smoking status
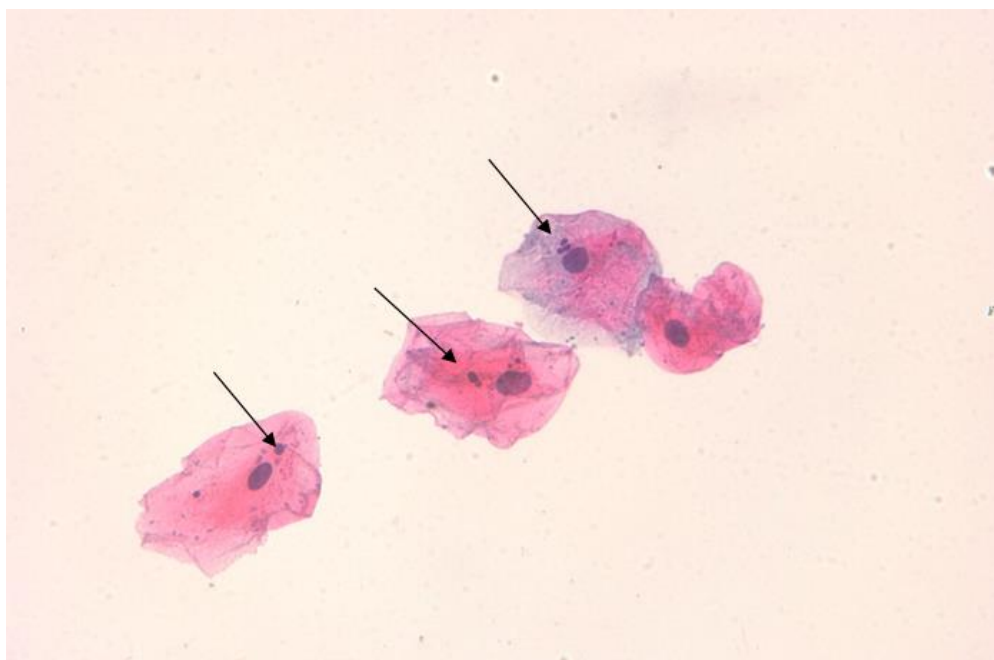

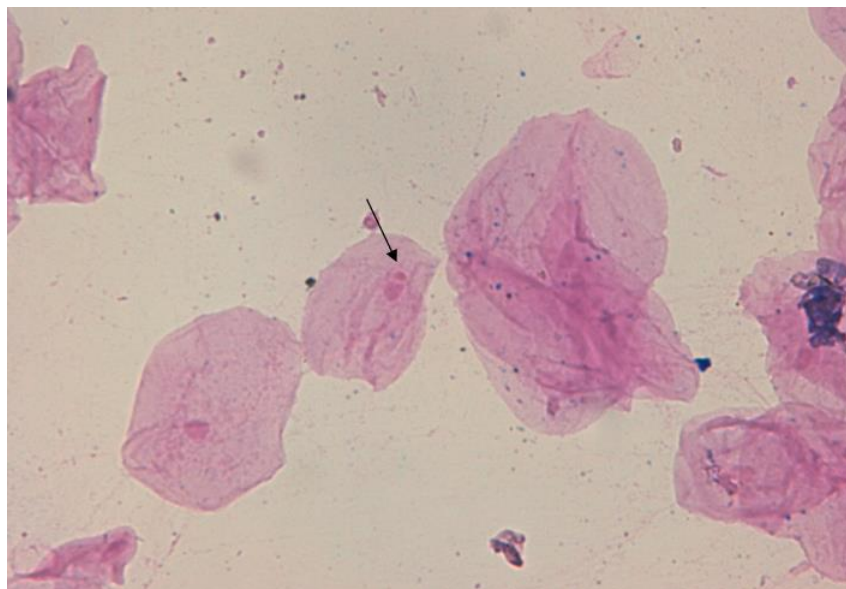**Figure 2:** Photomicrograph of a buccal cell with three Micronuclei (Pap Stain 400x).

Figure 2: Photomicrograph of a buccal cell show Micronuclei (Pap Stain 400x).
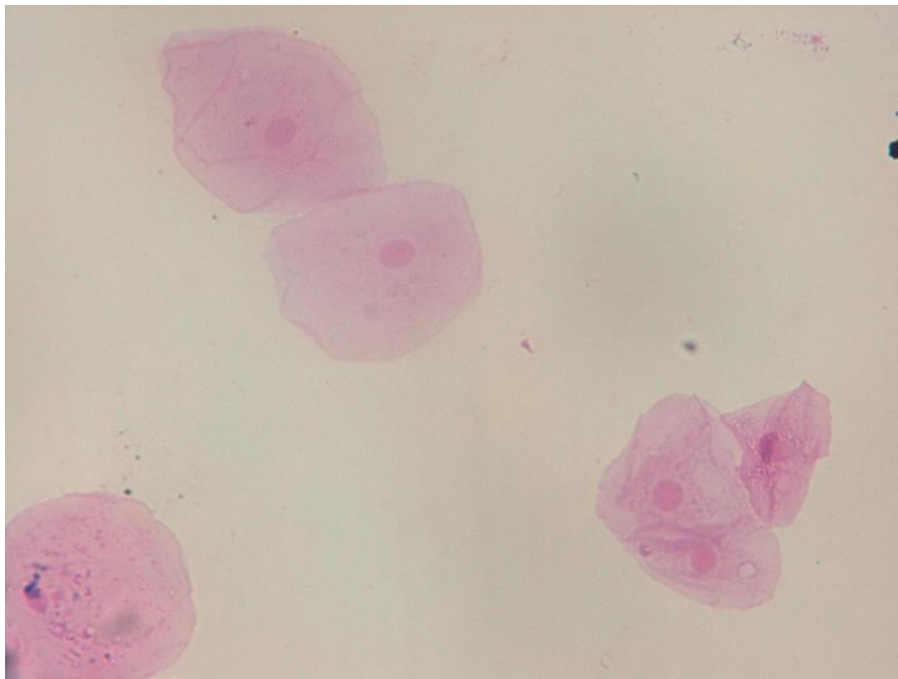
Figure 3: Photomicrograph of a buccal cell containing normal cells

4. Discussion

In vitro and in vivo studies have shown that a micronucleus test is a good way to find and measure the genotoxic effects of carcinogens and mutagens (6). It is about the same how sensitive the micronucleus test is as measuring chromatid breaks and exchanges. The micronucleus test was used on human cells that had been peeled off because there was a good link between drugs' ability to cause cancer and their ability to create micronuclei. The test was also easy to score, which made it more popular. The MN assay can be used on exfoliated cells, which is helpful because it lets a genotoxicity test be done on material from a whole organism, which has many defense systems already in place (6).

The results show that the percentage of MN cells was significantly higher in people who smoke compared to people who didn't smoke or use tobacco. The difference between the two groups was statistically highly significant. Recent studies by Sellapa et al (7) and Patel et al (8) also found that people who used smokeless tobacco had a higher MN count than people who didn't use smokeless tobacco. Compounds that cause cancer and mutations, such as tobacco-specific nitrosamines that are found in oral tobacco (39), are thought to be the cause of micronuclei. By using bacteria or enzymes, nicotine is turned into these substances. The same thing happens in the mouth when drool is present (9).

The micronucleus assay was used by Suhas et al. (9) to look at changes in buccal cells that are linked to smoking. They found that there was a strong link between smoking and the number of micronucleated mouth mucosal cells. Results from this study agree with those from this study.

Ozkul et al. (10) looked at micronuclei formation in the oral mucosal cells of people who regularly used Maras powder (a type of smokeless tobacco) and smokers. They found that the average percentage of micronucleated cells was 1.86+/- 0.26 in the users and 1.99+/-0.33 in the smokers. But there was no difference (p>0.05) between the two groups in the average number of micronucleated cells. The average number of micronucleated cells in people who smoke was 2.48 +/- 0.91, while it was 2.39 +/- 1.07 in people who smoked. There was a very big gap in the means between the two groups.

TSNAs, which are found in tobacco, have been linked to a higher chance of cancer in people who use smokeless tobacco (10). N-nitrosonornicotine (NNN), 4-methyl-N-nitrosamino-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT), and N-nitrosoanabasine (NAB) are the four main compounds. IARC (1985a) says that only two TSNAs, NNN and NNK, are likely to cause cancer. NAT and NAB, on the other hand, are not classifiable by IARC because they are not known to cause cancer (IARC, 1985b). In India, small businesses and individual farms process SLT, but they don't have much say over the fermentation and curing processes. This means that more TSNAs are made (11). Also, SLT isn't all the same in India because tobacco is often mixed with betel leaf (Piper betle), sliced areca nut (Areca catechu), and/or powdered slaked lime. These are additives that make tobacco more dangerous and increase its psychoactive effects (12).

Studies have also shown that Indian brands of smoking tobacco have a slightly higher nicotine content than foreign brands (13). While the nicotine level in widely available chewing tobacco is much lower than that in tobacco that is smoked, the amount of nicotine used each day makes it about the same as smoking tobacco (14).

The Center for Disease Control (CDC) says that chewing tobacco seven to eight times a day could be the same as smoking thirty to forty cigarettes a day. Another thing that made it easier for nicotine to get into the body was using slaked lime and staying in touch with the oral mucosa all the time. Also, people who chew tobacco absorbed nicotine through the oral mucosa and the gastrointestinal tract mucosa, while people who smoked nicotine absorbed it mostly through the pulmonary vasculature.

Chewing tobacco is said to have made it more likely to cause cancer and damage genes. People who smoke, drink, or chew tobacco have chances of 7.3 for getting oral cancer, 1.3 for drinking too much, and 11.4 for chewing tobacco (15).

5. Conclusion

The micronucleus test serves as a valuable tool for elucidating the genotoxic effects of tobacco use, providing insights into the mechanisms underlying tobacco - induced carcinogenesis and mutagenesis. The correlation between tobacco exposure and micronuclei formation in oral mucosal cells highlights the need for urgent action to address the global tobacco epidemic. By integrating the findings from MN assays into comprehensive tobacco control strategies, policymakers and public health authorities can work towards reducing the prevalence of tobacco use and preventing the associated burden of disease.

References

[1] World Health Organization. WHO report on the global tobacco epidemic, 2019: Offer help to quit tobacco use. World Health Organization; 2019. Available from: https: //www.who. int/publications/i/item/who - report - on - the - global - tobacco - epidemic - 2019_offer - help - to - quit - tobacco - use

[2] Fenech M, Kirsch - Volders M, Natarajan AT, Surralles J, Crott JW, Parry J, et al. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis.2011; 26 (1): 125–32. DOI: 10.1093/mutage/geq052

[3] Bonassi S, El - Zein R, Bolognesi C, Fenech M. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutat Res/Rev Mutat Res.2011; 711 (1–2): 1–6. DOI: 10.1016/j. mrrev.2010.12.003

[4] Abraham J, Mansoori NH, Nair P. Evaluation of micronuclei in exfoliated oral mucosal cells using Papanicolaou stain in normal, potentially malignant disorders and malignant lesions: A study on 50 cases. J Oral Maxillofac Pathol.2014; 18 (2): 169–75. DOI: 10.4103/0973 - 029X.140771

[5] Hayashi M, Sofuni T, Ishidate Jr. M. An application of acridine orange fluorescent staining to the micronucleus test. Mutat Res.1983; 120: 241–7.

[6] Sellappa S, Balakrishnan M, Raman S, Palanisamy S. Induction of micronuclei in buccal mucosa on chewing a mixture of betel leaf, areca nut and tobacco. J Oral Sci.2009; 51 (2): 289–92.

[7] Patel BP, Trivedi PJ, Brahmbhatt MM, Shukla SN, Shah PM, Bakshi SR. Micronuclei and chromosomal aberrations in healthy tobacco chewers and controls: A study from Gujarat, India. Arch Oncol.2009; 17 (1–2): 7–10.

[8] Hecht SS, Hoffmann D. Tobacco - specific nitrosamines. An important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis.1988; 9: 875–84.

[9] Winn DM, Blot WJ, Shy CM, Pickle XW, Toledo A, Fraumeni JF. Snuff dipping and oral cancer among women in the Southern United States. N Engl J Med.1981; 304: 745–8.

[10] Suhas S, Ganapathy KS, Gayatridevi, Ramesh C. Application of the micronucleus test to exfoliated epithelial cells from the oral cavity of beedi smokers, a high - risk group for oral cancer. Mutat Res.2004; 561: 15–21.

[11] Ozkul Y, Donmez H, Erenmemisoglu A, Demirtas H, Imamoglu N. Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users. Mutagenesis.1997; 12 (4): 285–7.

[12] Rodu B, Jansson C. Smokeless tobacco and oral cancer: A Review of the risks and determinants. Crit Rev Oral Biol Med.2004; 15 (5): 252–63.

[13] Main JH, Lecavalier DR. Smokeless tobacco and oral disease: A review. J Can Dent Assoc.1988; 54: 586–91.

[14] Sujatha SR, Reddy SHA. Estimation of nicotine content in popular Indian Brands of smoking and chewing tobacco products. Indian J Dent Res.2008; 19 (2): 88–91.

[15] Prabhakaran PS, Mani N. Epidemiology of oral cancer. Oral oncology CME annual. Kidwai Memorial Institute of Oncology: Bangalore; 2002. p.1–10