International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Cytological Alterations in Oral Mucosa Among Cigarette, Shisha, and E-Cigarette Users in Saudi Arabia: A Comparative Study

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Abstract: ***Tobacco use, including emerging alternatives like shisha and e-cigarettes, poses significant health risks beyond the systemic level. This study evaluates cytological alterations in the oral mucosa among users of cigarettes, shisha, and e-cigarettes, compared to nonsmokers. A total of 350 participants were examined using Papanicolaou staining of buccal smears, with cytological abnormalities assessed statistically. Results revealed significantly higher rates of abnormalities among smokers, with e-cigarette users exhibiting the most pronounced nuclear changes such as atypia and binucleation. The findings underscore the importance of oral cytology in early detection and highlight the urgent need for targeted smoking cessation policies.***

Keywords: Cytological abnormalities, oral mucosa, e-cigarette, shisha, cigarette smoking

1. Introduction

More than 8 million deaths worldwide are attributed to tobacco use each year, including those of direct smokers and those exposed to second hand smoke [1]. In addition to its well-established systemic consequences, which include cancer, chronic respiratory disorders, and cardiovascular disease, tobacco smoking has significant local effects on the oral cavity. As the main site of exposure, the oral mucosa is especially susceptible to the thermal and chemical insults brought on by tobacco use [2].

Gingival inflammation, periodontal disease, delayed wound healing, increased plaque accumulation, oral malodor, and tooth and mucosal discoloration are among the oral health effects of smoking [3]. Furthermore, tobacco smoking is known to be a significant risk factor for oral squamous cell carcinoma (OSCC) and has been linked to the pathophysiology of premalignant lesions such leukoplakia and erythroplakia [4,5]. At least 70 recognized carcinogens, including formaldehyde, nitrosamines, and polycyclic aromatic hydrocarbons, are among the complex combination of more than 7,000 compounds found in cigarette smoke. These chemicals cause and encourage mutagenesis in oral epithelial cells [6].

Alternative tobacco use methods, such shisha (waterpipe) and e-cigarettes (electronic cigarettes), have become more popular in recent years, especially among younger people in Asia and the Middle East [7]. Despite data to the contrary, the false belief that these substitutes are less dangerous than traditional cigarettes has resulted in a rise in consumption. For example, waterpipe smoke is frequently inhaled in larger quantities over extended periods of time, yet it has identical harmful components to cigarette smoke [8]. Despite not producing any combustion products, e-cigarettes contain nicotine and other flavorings that have been demonstrated to have cytotoxic and inflammatory effects on oral tissues [9].

According to histopathology, exposure to tobacco smoke, in any form, has been linked to notable cellular alterations in the oral mucosa. These include keratinization, basal cell hyperactivity, epithelial hyperplasia, and altered expression of proteins that regulate apoptosis and cell proliferation [10]. To evaluate these alterations and detect early neoplastic transformation, biomarkers such the tumor suppressor gene p53, the proliferation marker Ki-67, and the anti-apoptotic protein Bcl-2 have been widely used [11,12]. Smokers' oral mucosal tissues have been found to have elevated Bcl-2 expression and Ki-67 labeling indices, which frequently correlate with histological degrees of dysplasia [13]. These molecular changes may occur before lesions become clinically apparent, highlighting their potential use in risk assessment and early detection.

Comparative analyses of various smoking modalities and their distinct effects on the oral epithelium are still few, despite the fact that the effects of cigarette smoking on oral tissues have been the subject of countless research. Understanding how each type of smoking has different effects is essential given the variety of tobacco products and user exposure patterns. The purpose of this study is to examine and contrast the cytological alterations in the oral mucosa and the state of oral health among users of different tobacco products, such as e-cigarettes, waterpipes, and cigarettes. This study aims to clarify the range of tobacco-induced oral alterations and add to the expanding body of data required to guide clinical screening methods and public health policies by employing immunohistochemistry markers and histological evaluations.

By identifying early cytological changes linked to various smoking types, this study contributes critical insights for early oral cancer detection and targeted prevention strategies

2. Materials and Methods

Study Design and Participants From:
From January to May 2025, 350 randomly selected healthy volunteers participated in this cross-sectional study, including 300 cigarette smokers and 50 nonsmokers serving as the control group. The Epi Info Software Package Version 7.2 (Centers for Disease Control and Prevention, Atlanta, Georgia) determined the sample size based on a 95% confidence level and a 5% margin of error. All participants provided two buccal smears each, with the study adhering strictly to safety protocols. The study included Saudi nationals aged 18 to 85 years who were in good general health, regardless of smoking status. Exclusion criteria included non-Saudi citizens and individuals under the age of 18.

Sample Collection:
Buccal smears were collected using wooden tongue depressors from the tongue dorsum and both cheeks. Using a wooden tongue depressor, we obtained exfoliative cells from the oral mucosa, specifically from the tongue dorsum and both cheeks. Cells were evenly spread on two clean glass slides and immediately fixed in 95% ethyl alcohol in 95% ethyl alcohol while they were still damp. We dispatched the buccal smears to the histopathology lab at Rayyan College of Medicine in Saudi Arabia for staining and diagnosis.

Papanicolaou's Staining:
After fixation in ethanol, we hydrated the smears in a descending series of ethanol concentrations (diluted with distilled water) from 95% to 70% for two minutes each. We treated the smears with Harris hematoxylin for five minutes to stain the nuclei, rinsed them in distilled water, differentiated them in 0.5% aqueous hydrochloric acid for ten seconds, and then rinsed them again in distilled water. After blueing in alkaline water for four seconds in alkaline water, we dehydrated the smears twice, for two minutes each, using an ascending series of ethanol concentrations from 70% to 95%. We then stained the smears with Papanicolaou Orange G6 solution for two minutes, rinsed them with 95% ethanol, incubated them with Papanicolaou EA50 staining solution for three minutes, and checked for cytoplasmic staining. Following dehydration in 95% pure ethanol, we cleared the smears in xylene and mounted them using dibutylphthalate polystyrene xylene (DPX) [17].

Cytological Evaluation:
We examined Pap-stained smears for cytopathological abnormalities. These smears were examined for signs of keratinization, inflammation, infection, and cellular atypia. Cytological changes identify features such as uneven growth and bi- or multi-nucleation [9].

Quantitative Analysis:
We used IBM SPSS Statistics for Windows, Version 22 (published in 2013; IBM Corp., Armonk, New York), for statistical analysis, setting the significance level at 0.05. We represented categorical data as frequencies or proportions and examined the study topics and data types with chi-square testing.

Ethical Consent:
Before collecting specimens, each participant was required to complete a written ethical consent form. The Al Rayyan Medical Colleges (AMC) Ethical Committee designed and approved the informed ethical consent form.

3. Results

Cytological Findings
Out of the 300 smokers, 144 (48%) showed cytological abnormalities, including inflammatory cells, microbial infections, cellular atypia, and binucleated/multinucleated epithelial cells. In contrast, among the 50 nonsmokers, only 10 (20%) exhibited similar abnormalities. This distribution is detailed in Table 1 below.

Table 1: Study group and cytological findings

Group	Normal Cells (%)	Abnormal Cells (%)
Smokers (n=300)	156 (52%)	144 (48%)
Nonsmokers (n=50)	40 (80%)	10 (20%)

The majority of smokers were in the younger age groups (18–45), while nonsmokers were more evenly distributed across all age groups. The highest concentration of smokers was observed in the 18–30 age group (n = 90) as shown in Figure 1

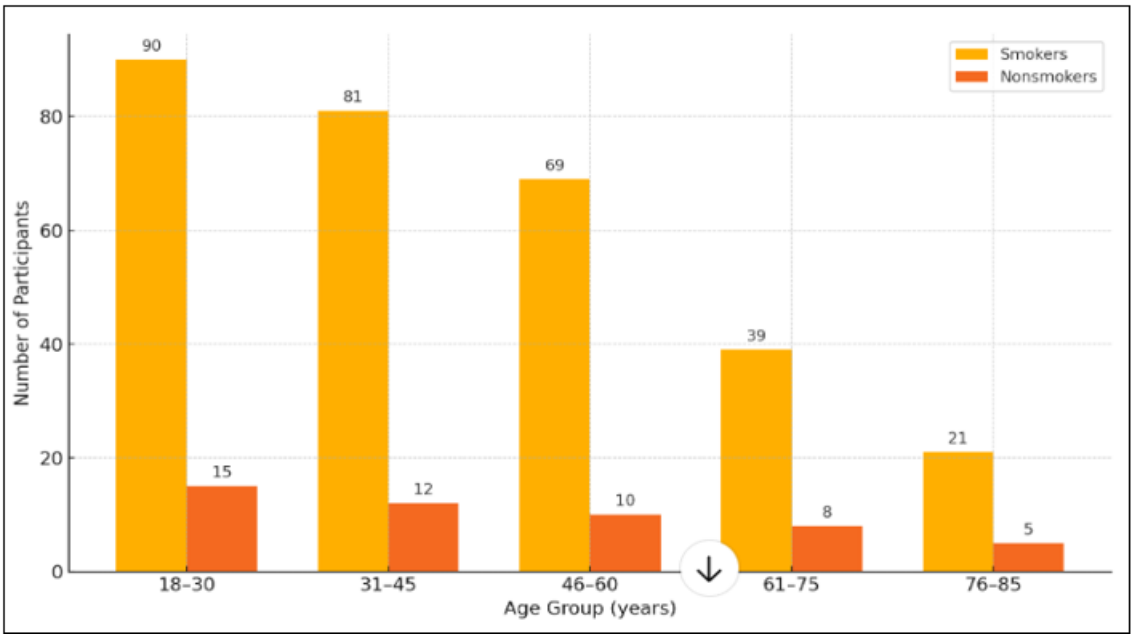

Figure 1: Age Distribution of Smokers and Nonsmokers (N = 350)

Males were predominant in both groups, with 72% of smokers and 68% of nonsmokers being male. The gender distribution difference between smokers and nonsmokers was not statistically significant (P = 0.27) as shown in Figure 2

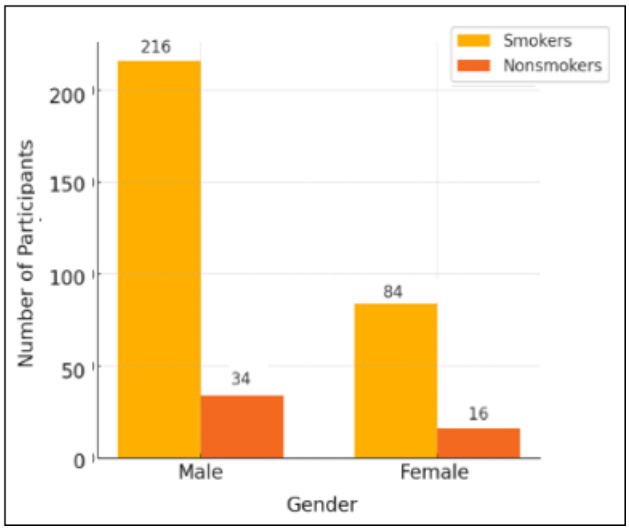

Figure 2: Age Distribution of Smokers and Nonsmokers (N = 350)

Among the 300 smokers, participants were categorized into three subgroups: cigarette smokers (n = 120), shisha smokers (n = 100), and e-cigarette users (n = 80). The prevalence of cytological abnormalities varied among the groups: 52 (43.3%) in cigarette smokers, 48 (48%) in shisha smokers, and 44 (55%) in e-cigarette users. In contrast, among the 50 nonsmokers, only 10 (20%) exhibited cytological abnormalities. The difference was statistically significant (P = 0.023) Table 2.

Table 2: Cytological Findings by Smoking Type

Group	Normal Cells (n, %)	Abnormal Cells (n, %)	Total (n)
Cigarette Smokers	68 (56.7%)	52 (43.3%)	120
Shisha Smokers	52 (52%)	48 (48%)	100
E-Cigarette Users	36 (45%)	44 (55%)	80
Nonsmokers	40 (80%)	10 (20%)	50

Among the 300 smokers (120 cigarette smokers, 100 shisha smokers, 80 e-cigarette users), a variety of cytological abnormalities were observed. These included inflammatory cells, infectious changes, cellular atypia, and binucleated/multinucleated cells. The highest frequency of atypia and binucleation was found in e-cigarette users, while inflammation was more prevalent among cigarette smokers. The breakdown is shown in Table 3

Table 3: Cytological Abnormalities by Smoking Type

Abnormality Type	Cigarette Smokers (n = 120)	Shisha Smokers (n = 100)	E-Cigarette Users (n = 80)
Inflammatory Cells	28 (23.3%)	22 (22%)	15 (18.8%)
Infection (e.g., Candida)	10 (8.3%)	12 (12%)	10 (12.5%)
Cellular Atypia	8 (6.7%)	9 (9%)	12 (15%)
Binucleated/Multinucleated Cells	6 (5%)	5 (5%)	7 (8.8%)

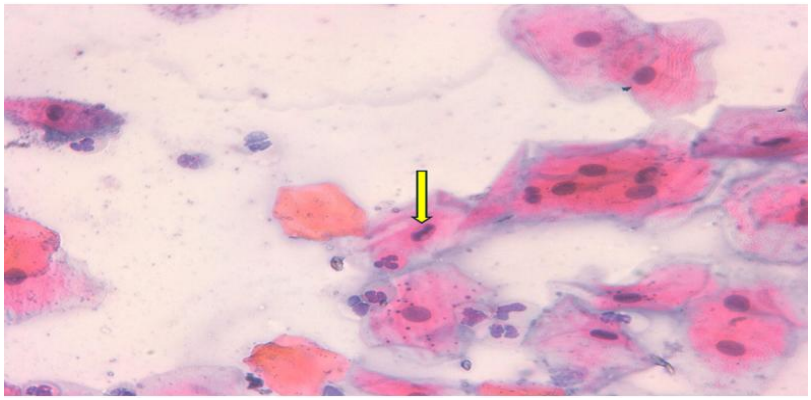

Figure 3: Microphotographs of smear samples from buccal mucosa stained with Papanicolaou's method (x40) demonstrate Atypia (Shisha Smokers)

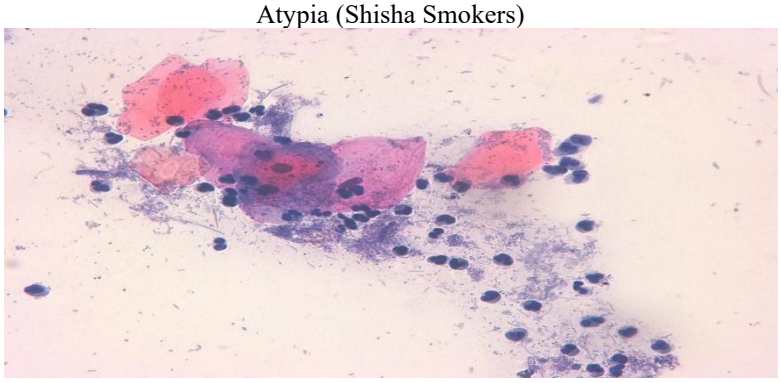

Figure 4: Microphotographs of buccal smears from cigarette smokers with Pap staining (x40) show the inflammatory cells

4. Discussion

This study showed that cytological abnormalities in the oral mucosa are substantially correlated with smoking, independent of the kind of smoking. Among the 300 smokers evaluated, 48% showed cytological abnormalities, whereas only 20% of nonsmokers did. These anomalies included binucleated or multinucleated epithelial cells, cellular atypia, microbial infections, and inflammatory infiltrates. According to the results, smoking has a significant role in the early cytopathological alterations of the oral epithelium, which is a crucial stage in the development of malignancy [14].

Cytological abnormalities were most common among e-cigarette users (55%), shisha smokers (48%), and cigarette smokers (43.3%), according to the research. Interestingly, e-cigarette users were more likely to experience atypia and binucleation, whereas cigarette smokers were more likely to experience inflammatory alterations. These results are consistent with those of Salih et al., who found that the cytological severity increased with the length of smoking for 51.4% of traditional cigarette users and 37.5% of e-cigarette users [15]. Significantly higher micronuclei counts were also reported by Pop et al. and Elmahdi in smokers, indicating chromosomal instability and nuclear damage—early indicators of dysplasia [16,17].

The epidemiological risk is supported by the observed distribution by gender and age. Our study's majority of smokers were young people between the ages of 18 and 30, which is consistent with national statistics showing an increase in the use of e-cigarettes and waterpipes among Saudi youth [18]. Although men were more likely than women to smoke or not, the gender gap was not statistically significant ($P = 0.27$).

Users of e-cigarettes showed the highest frequency of binucleated cells (8.8%) and cellular atypia (15%) in terms of particular cytological features, indicating significant nuclear disruption despite lower perceived toxicity. This is in line with Pop et al.'s findings that e-cigarette users had micronuclei counts of 3.21 ± 1.12 per 1000 cells, which is equivalent to cigarette smokers' values of 3.6 ± 1.08 [16]. Nicotine's metabolites, such as nitrosamines, and other e-liquid ingredients, such as propylene glycol and glycerin, are known to induce oxidative stress and damage to the epithelium, even though nicotine itself is not a direct carcinogen [19].

Conversely, Shisha smokers had mild atypia (9%), and higher rates of infection-related abnormalities (12%). Our study confirmed the findings of Seifi et al., who reported that waterpipe users had inflammation, increased nuclear size, and a nuclear-cytoplasmic ratio [20]. Additionally, Abdul et al. found that waterpipe users had substantially greater amounts of micronuclei (1.94 ± 0.39) than nonsmokers (1.68 ± 0.35), which aligns with our findings suggesting that hookah use may contribute to chromosomal damage [21].

Direct chemical irritation from combustion byproducts is probably the cause of the high occurrence of inflammation among cigarette smokers (23.3%). The degree of cytological damage was still significant even though the overall rate of atypia was somewhat lower in these smokers. Salih et al. have previously noted a dose-dependent connection, with reactive alterations rising from 33.8% in smokers with less than five years of exposure to 71.8% in those with more than five years [15].

Oral exfoliative cytology is a useful non-invasive method for screening high-risk people, especially smokers, according to the study's findings. Particularly in young users of new products like e-cigarettes, cytological alterations such micronuclei development, atypia, and binucleation can serve as early warning indicators of precancerous transformation.

5. The Limitations

The study explores the cytological effects of different smoking types, but has limitations such as a cross-sectional design, small nonsmoker sample size, inability to control factors like smoking frequency, years of exposure, oral hygiene practices, alcohol use, and systemic health conditions, and the reliance on exfoliative cytology without histopathological confirmation.

6. Conclusions

This study reveals a clear association between smoking—particularly e-cigarette use—and cytological abnormalities in the oral mucosa. These alterations, including cellular atypia and binucleation, signal early precancerous changes and underscore the utility of exfoliative cytology for screening. The findings call for urgent public health attention toward emerging smoking trends and reinforce the need for preventive education targeting youth populations.

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