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Cytological Alterations and E-Cadherin Expression in the Oral Mucosa of Shisha Smokers in Saudi Arabia

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Abstract: ***Background: Shisha smoking is a prevalent form of tobacco use in Saudi Arabia and is a significant risk factor for oral cancer. Tobacco exposure alters cellular processes such as adhesion, proliferation, and differentiation. E-cadherin, a key cell adhesion molecule, plays a critical role in maintaining epithelial integrity, and its dysregulation may contribute to oral mucosal pathology. Objective: This study aims to assess cytological changes and the immunohistochemical expression of E-cadherin in the oral mucosa of Saudi shisha smokers. Methods: A cross-sectional study was conducted from March to June 2025, involving 200 buccal mucosa samples from Saudi participants of both genders. The study included 100 shisha smokers and 100 non-smokers as controls. Samples were collected using exfoliative cytology techniques. Immunohistochemical staining was performed to evaluate E-cadherin expression by assessing staining intensity and the percentage of positively stained cells. Data analysis was conducted using SPSS, with categorical variables expressed as frequencies and percentages. The chi-square test was used to assess associations, considering P<0.05 as statistically significant. Results: Cytological abnormalities such as cellular atypia, inflammation, and binucleation were significantly more frequent in shisha smokers (46%) compared to non-smokers (18%) (P-0.012). A marked increase in positive E-cadherin expression was observed in the oral mucosa of shisha smokers (23%) compared to non-smokers (3%) (P-0.005). No significant differences in E-cadherin expression were found across age groups (P-0.48) or gender (P-0.21). Conclusion: The findings highlight that shisha smoking induces significant cytological alterations and downregulation of E-cadherin in the oral mucosa, suggesting early disruption of epithelial integrity and increased risk for malignant transformation. These results emphasize the need for public health measures targeting shisha use and the potential of E-cadherin as a biomarker for early oral mucosal changes in smokers.***

Keywords: Cytological changes, Oral mucosa, E-cadherin, Shisha smokers, Saudi Arabia

1. Introduction

Tobacco smoking is a well-established risk factor for oral cancer, contributing significantly to morbidity and mortality worldwide. In recent years, shisha (waterpipe) smoking has gained popularity, particularly in Middle Eastern countries such as Saudi Arabia, where it is widely used among males [1,2]. Although shisha smoking is often perceived as less harmful than cigarette smoking, research indicates that it exposes users to similar or even higher levels of carcinogens, including polycyclic aromatic hydrocarbons, heavy metals, and nicotine [3,7].

Cytological studies of the oral mucosa in shisha smokers reveal increased incidence of cellular alterations such as inflammation, infection, cellular atypia, and keratinization, which are indicative of early mucosal damage and potential premalignant changes. Elmahdi et al. demonstrated that Saudi male shisha smokers exhibited significantly higher rates of these cytological abnormalities compared to non-smokers [1]. These morphological changes reflect the chronic irritation and genetic damage induced by tobacco-related toxins.

Immunohistochemical analyses further highlight molecular disruptions in the oral mucosa of shisha smokers. The tumor suppressor protein p53, which plays a crucial role in DNA repair and apoptosis, is frequently overexpressed in the mucosal tissues of smokers, correlating with dysplastic and malignant transformations [2,4]. Similarly, increased expression of Ki-67, a marker of cellular proliferation, has been reported, indicating enhanced mitotic activity in these tissues [1]. These biomarkers suggest that shisha smoking contributes to oncogenic processes at the cellular level.

E-cadherin, a calcium-dependent adhesion molecule, is essential for maintaining epithelial cell-cell adhesion and tissue architecture. Loss or downregulation of E-cadherin expression is a hallmark of epithelial-mesenchymal transition (EMT), which facilitates cancer cell invasion and metastasis [5,6]. However, despite its recognized role in carcinogenesis, there is a notable absence of data regarding E-cadherin immunoexpression in the oral mucosa of shisha smokers, representing a critical gap in current knowledge.

Given the increasing prevalence of shisha smoking in Saudi Arabia and the associated risk of oral cancer, it is imperative to investigate both cytological alterations and molecular markers such as E-cadherin. This study aims to assess these changes to better understand the early events of oral carcinogenesis linked to shisha use in this population.

2. Materials and Methods

Study Design and Participants From:
Between March and June of 2025. In this cross-sectional study, 200 healthy volunteers were chosen at random; 100 of them were shisha smokers, while the other 100 served as the control group. Every subject gave two buccal swabs, and the study closely followed safety procedures. Regardless of whether they smoked or not, Saudi citizens between the ages of 18 and 85 who were in good overall health were included

in the study. Those under the age of eighteen and non-Saudi nationals were excluded.

Sample Collection:
Buccal smears were collected by gently scraping the inner cheek and tongue surfaces for cellular analysis. We extracted exfoliative cells from the oral mucosa, namely from the dorsum of the tongue and both cheeks, using a wooden tongue depressor. We then spread the cells uniformly across two clean glass slides and immediately preserved them in 95% ethyl alcohol while they were still wet. For staining and diagnosis, we sent the buccal smears to the Rayyan College of Medicine's histopathology lab in Saudi Arabia.

Papanicolaou's Staining:
Following ethanol fixation, we hydrated the smears for two minutes at a time in a decreasing order of ethanol concentrations (diluted with distilled water), ranging from 95% to 70%. After staining the nuclei for five minutes with Harris hematoxylin, we washed the smears in distilled water, differentiated them for ten seconds in 0.5% aqueous hydrochloric acid, and then rinsed them once more in distilled water. We used an escalating sequence of ethanol concentrations from 70% to 95% to dehydrate the smears twice, for two minutes each, after a four-second blue coloring procedure in alkaline water. After two minutes of staining with Papanicolaou Orange G6 solution, we washed the smears with 95% ethanol, incubated them for three minutes with Papanicolaou EA50 staining solution, and looked for cytoplasmic staining. After the smears were dehydrated in 95% pure ethanol, we cleaned them in xylene and used dibutylphthalate polystyrene xylene (DPX) to mount them [11].

Immunocytochemistry:
We used phosphate-buffered saline (PBS) to rinse the smears three times, each lasting three minutes. We treated each slide with a 0.3% hydrogen peroxide in methanol solution for 15 minutes in order to reduce the activity of endogenous peroxidase. After that, we used PBS to rinse the slides three times. The slides were then incubated for 30 minutes at 37 °C with primary mouse monoclonal E-cadherin antibodies (Gene Tech Company Limited, Shanghai, China) at a dilution of 1/100. we applied a secondary HRP-conjugated antibody (Chem Mate EnVision+ system) (Gene Tech Company Limited), to the slides and let them to sit at room temperature for half an hour. Three PBS washes were then conducted, and two more were conducted after that. To quantify immunoreactivity, we employed Gene Tech Company Limited's diaminobenzidine (DAB) at a dilution of 1/100. After applying the chromogen for ten minutes, we gave it a three-minute wash with distilled water. Finally, we stained the slices with hematoxylin counterstain for three minutes. After rinsing the parts for five minutes under running tap water, we dehydrated them using a series of alcoholic solutions. We then used xylene to clean the parts before mounting them with DPX.

Interpretation Criteria: Cytoplasmic expression patterns were used to assess E-cadherin staining. The presence of a distinct brown hue in the stained cells' cytoplasm indicated positive staining for both markers [9].

Cytological Evaluation:
We looked for cytopathological anomalies in Pap-stained smears. We searched for signs of infection, inflammation, keratinization, and atypia. Features like bi- or multi-nucleation and uneven development are identified by cytological alterations [9].

Quantitative Analysis:
For statistical analysis, we established the significance threshold at 0.05 and utilized IBM SPSS Statistics for Windows, Version 22 (released in 2013; IBM Corp., Armonk, New York). We used chi-square testing to analyze the research themes and data types, and we expressed categorical data as frequencies or proportions.

Ethical Consent:
Each participant has to fill out a documented ethical approval form before to specimen collection. The informed ethical consent form was created and authorized by the Al Rayyan Medical Colleges (AMC) Ethical Committee.

3. Results

Cytological Findings

The study included 200 participants, divided equally into shisha smokers (n-100) and non-smokers (n-100). The mean age of shisha smokers was 28.4 ± 6.2 years, and non-smokers had a mean age of 29.1 ± 5.8 years, with no statistically significant difference (p - 0.42). Age group distribution was similar between groups, with the majority in the 26–35 age range. Most participants were male in both groups (shisha smokers: 85%, non-smokers: 82%), reflecting the higher prevalence of shisha smoking among males in Saudi Arabia (p - 0.58) as shown in Table 1.

Table 1: Demographic Characteristics of Study Participants

Characteristic	Shisha Smokers (n-100)	Non-Smokers (n-100)
Age (years), mean ± SD	28.4 ± 6.2	29.1 ± 5.8
Age group (years)		
18–25	30 (30%)	28 (28%)
26–35	45 (45%)	50 (50%)
36–45	15 (15%)	14 (14%)
46+	10 (10%)	8 (8%)
Gender		
Male	85 (85%)	82 (82%)
Female	15 (15%)	18 (18%)

A total of 200 participants were enrolled, comprising 100 shisha smokers and 100 non-smokers. Cytological examination revealed significant differences in the prevalence of abnormalities between the two groups (Table 2). Shisha smokers exhibited higher rates of inflammation, infection, cellular atypia, and keratinization compared to non-smokers ($p < 0.05$).

Table 2: Cytological Changes in Oral Mucosa of Shisha Smokers and Non-Smokers

Parameter	Shisha Smokers (n-100)	Non-Smokers (n-100)	p-value
Inflammation (%)	46 (46%)	18 (18%)	0.04
Infection (%)	12 (12%)	5 (5%)	
Cellular Atypia (%)	10 (10%)	2 (2%)	
Keratinization (%)	8 (8%)	1 (1%)	

Shisha smokers showed significantly higher positive E-cadherin expression (23%) compared to non-smokers (3%) (p - 0.005). Negative expression was more common in non-smokers (97%) than smokers (73%). This indicates altered cell adhesion in shisha smokers as shown in Table 3.

Table 3. Comparison of E-cadherin Expression Between Shisha Smokers and Non-Smokers

Parameter	Shisha Smokers (n-100)	Non-Smokers (n-100)	p-value
Positive E-cadherin (%)	23(23%)	3 (3%)	0.005
Negative E-cadherin (%)	73 (73%)	97 (97%)	

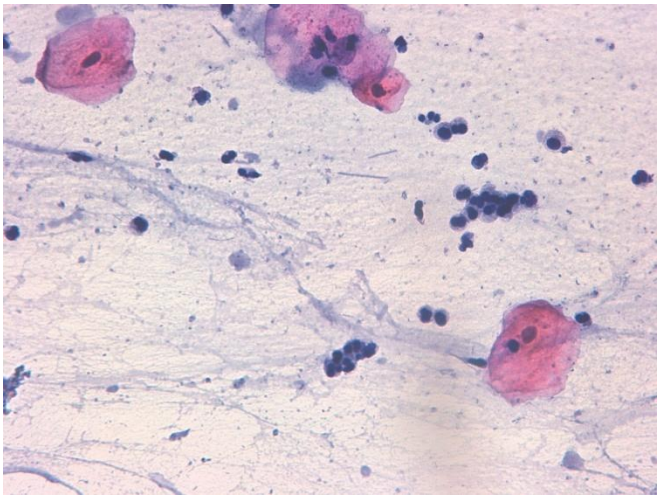

Figure 1: Microphotographs of smear samples from buccal mucosa stained with Papanicolaou's method (x40) demonstrate inflammatory cells

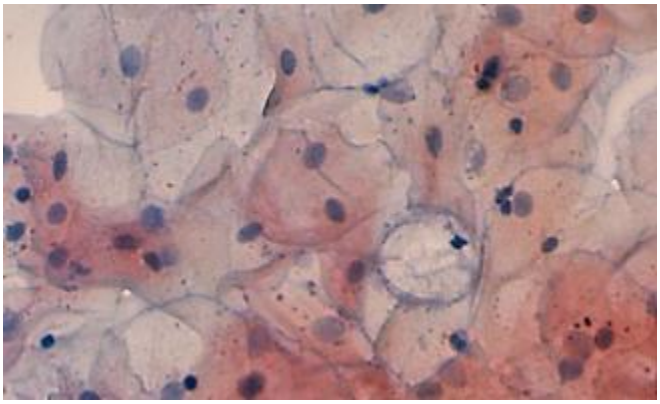

Figure 2: Microphotographs of buccal smears from cigarette smokers with immunohistochemical staining (x40) show the expression of E-cadherin in the brown color of the cytoplasm

4. Discussion

This study highlights significant cytological alterations in the oral mucosa of shisha smokers compared to non-smokers, reflecting the harmful effects of waterpipe tobacco on epithelial cells. The increased incidence of inflammation, infection, cellular atypia, and keratinization in shisha smokers supports existing evidence that tobacco smoke exposure induces chronic irritation and cellular damage in the oral cavity [12,13]. These cytological changes are early indicators of mucosal injury and may represent precancerous alterations that increase the risk of oral malignancies in chronic users.

Immunohistochemical analysis revealed a significantly higher positive expression of E-cadherin in shisha smokers (23%) compared to non-smokers (3%). E-cadherin, a key molecule in cell-cell adhesion, plays a pivotal role in maintaining epithelial tissue integrity, and its aberrant expression is linked to carcinogenesis through processes like epithelial-mesenchymal transition (EMT) [14,15]. The elevated positive staining in smokers could suggest a cellular attempt to maintain adhesion in the face of tobacco-induced stress, possibly reflecting a compensatory mechanism during early epithelial disruption.

Contrasting with the classical view of E-cadherin downregulation in established cancers, our findings indicate that alterations in E-cadherin expression may begin in preclinical stages of mucosal damage, as seen in smokers without overt lesions. This aligns with recent studies proposing that dysregulated E-cadherin expression in tobacco-exposed mucosa might serve as an early biomarker of epithelial transformation risk [16,17]. The relative preservation or even increase in E-cadherin positivity among smokers could represent a complex regulatory response preceding malignant progression.

No significant variation in E-cadherin expression was noted across different age groups or between genders in this cohort, emphasizing that tobacco exposure itself is the primary determinant of molecular changes in the oral epithelium rather than demographic factors. Given the high prevalence of shisha use among young adults and males in Saudi Arabia, these findings stress the importance of targeting these populations with preventive strategies and education to reduce the burden of tobacco-related oral diseases [18].

5. Conclusions

In conclusion, our study underscores the role of shisha smoking in inducing both cytological abnormalities and molecular alterations in oral mucosa, particularly affecting E-cadherin expression. These changes likely contribute to early epithelial disruption and increased susceptibility to oral carcinogenesis. Public health initiatives aimed at reducing shisha use and incorporating molecular screening tools such as E-cadherin immunoexpression could be valuable in the early detection and prevention of oral cancer in high-risk populations.

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