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Immunohistochemical of E-Cadherin Expression in the Oral Mucosa of Saudi Smokers and Non-Smokers

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Abstract: ***Background: Smoking is a common habit in Saudi Arabia and a recognized risk factor for oral cancer. Tobacco exposure disrupts normal epithelial cell behavior, particularly adhesion, proliferation, and differentiation. E-cadherin, a vital cell adhesion molecule, maintains epithelial integrity, and its reduced expression may contribute to oral mucosal pathology. Objective: To evaluate the immunohistochemical expression of E-cadherin and associated cytological changes in the oral mucosa of Saudi smokers compared with non-smokers. Methods: This cross-sectional study, conducted between March and June 2025, included 200 buccal mucosa samples from Saudi adults (100 smokers and 100 non-smokers). Samples were collected using exfoliative cytology. Immunohistochemical staining for E-cadherin was performed, and expression was assessed based on staining intensity and the percentage of positive cells. Data were analyzed using SPSS; categorical variables were summarized as frequencies and percentages, and associations were tested using the chi-square test, with P<0.05 considered significant. Results: Cytological abnormalities-including cellular atypia, inflammation, and binucleation-were significantly more frequent among smokers (46%) than non-smokers (18%) (P=0.012). E-cadherin expression was markedly reduced in smokers (15%) compared with non-smokers (6%) (P=0.025). No significant variation in E-cadherin expression was observed across age (P=0.48) or gender (P=0.21). Conclusion: Smoking is associated with significant cytological alterations and reduced E-cadherin expression in the oral mucosa, indicating early epithelial disruption and a potential predisposition to malignant transformation. These findings underscore the importance of public health initiatives to reduce tobacco use and highlight E-cadherin as a potential biomarker for early detection of smoking-induced oral changes.***

Keywords: E-cadherin, Oral mucosa, Cytological changes, Smoking, Immunohistochemistry, Saudi population

1.Introduction

Tobacco smoking is a well-established risk factor for oral cancer, contributing substantially to global morbidity and mortality. In recent years, smoking has become increasingly prevalent in Middle Eastern countries such as Saudi Arabia, particularly among males [1,2]. Despite the widespread misconception that certain forms of smoking, such as waterpipe use, are less harmful than cigarettes, evidence shows that smokers are exposed to comparable or even higher concentrations of carcinogens, including polycyclic aromatic hydrocarbons, heavy metals, and nicotine [3,7].

Cytological investigations of the oral mucosa in smokers have revealed a higher incidence of cellular abnormalities such as inflammation, infection, cellular atypia, and keratinization, all of which indicate early mucosal injury and potential precancerous transformation. Elmahdi et al. reported that Saudi male smokers demonstrated significantly elevated rates of these cytological alterations compared to non-smokers [1]. These morphological changes reflect the chronic irritation and genotoxic stress induced by tobacco-derived toxins.

Immunohistochemical studies have further demonstrated molecular disturbances within the oral mucosa of smokers. The tumor suppressor protein p53, which regulates DNA repair and apoptosis, is frequently overexpressed in smokers' mucosal tissues, correlating with dysplastic and malignant transformation [2,4]. Likewise, the proliferative marker Ki-67 is often elevated, indicating increased cellular turnover and proliferative activity [1]. Together, these findings suggest that smoking triggers molecular pathways contributing to oncogenesis at the epithelial level.

E-cadherin, a calcium-dependent cell adhesion molecule, plays a critical role in maintaining epithelial integrity and intercellular cohesion. Downregulation or loss of E-cadherin expression is a key event in epithelial-mesenchymal transition (EMT), a process that facilitates cancer invasion and metastasis [5,6]. Despite its recognized role in carcinogenesis, limited research has examined E-cadherin immunoexpression in the oral mucosa of smokers, leaving a gap in understanding how tobacco exposure affects early molecular events in epithelial pathology.

Given the high prevalence of smoking in Saudi Arabia and its established link to oral malignancy, it is essential to investigate both cytological and molecular alterations within the oral mucosa. This study aims to evaluate E-cadherin immunoexpression and cytological changes in the oral mucosa of Saudi smokers compared with non-smokers, to provide insight into the early cellular and molecular mechanisms underlying tobacco-related oral carcinogenesis.

2.Materials and Methods

Study Design and Participants

This cross-sectional study was conducted between March and June 2025 and included 200 healthy Saudi volunteers selected randomly. Among them, 100 were cigarette smokers, while the remaining 100 non-smokers served as the control group. Each participant provided two buccal swab samples, and all procedures adhered to standard biosafety protocols.

Eligible participants were Saudi citizens aged 18 to 85 years in good general health. Exclusion criteria included individuals under 18 years of age and non-Saudi nationals.

Sample Collection

Buccal smear samples were collected to obtain exfoliated epithelial cells from the oral mucosa. Using a sterile wooden tongue depressor, cells were gently scraped from the dorsum of the tongue and the inner surfaces of both cheeks. The collected material was evenly spread onto two clean glass slides and immediately fixed in 95% ethyl alcohol while still wet. All prepared smears were submitted to the Histopathology Laboratory at Al-Rayan College of Medicine, Madinah, Saudi Arabia, for staining and analysis.

Papanicolaou Staining

After ethanol fixation, the smears were rehydrated in descending ethanol concentrations (95% to 70%) for two minutes each. The nuclei were stained with Harris hematoxylin for five minutes, followed by washing in distilled water and differentiation for 10 seconds in 0.5% aqueous hydrochloric acid. The slides were then rinsed again in distilled water and blued in alkaline water for four seconds.

Dehydration was performed through ascending ethanol concentrations (70% to 95%), each for two minutes. Cytoplasmic staining was achieved using Papanicolaou Orange G6 solution for two minutes, followed by a 95% ethanol rinse and subsequent staining with Papanicolaou EA50 solution for three minutes. After dehydration in 95% ethanol, the smears were cleared in xylene and mounted using dibutylphthalate polystyrene xylene (DPX) [11].

Immunocytochemistry for E-Cadherin

Smears were rinsed three times in phosphate-buffered saline (PBS) for three minutes each. To block endogenous peroxidase activity, slides were treated with 0.3% hydrogen peroxide in methanol for 15 minutes, then rinsed again in PBS.

Primary mouse monoclonal E-cadherin antibody (Gene Tech Company Limited, Shanghai, China) was applied at a dilution of 1:100 and incubated at 37 °C for 30 minutes. After washing with PBS, a secondary antibody (ChemMate™ EnVision+/HRP, Gene Tech Company Limited) was added and incubated at room temperature for 30 minutes. The reaction was visualized using diaminobenzidine (DAB, Gene Tech Company Limited) at a 1:100 dilution for 10 minutes, followed by a distilled water rinse. Slides were counterstained with hematoxylin for three minutes, rinsed under running tap water, dehydrated through graded alcohols, cleared in xylene, and mounted with DPX.

Interpretation Criteria

E-cadherin expression was evaluated based on cytoplasmic staining patterns. The presence of a distinct brown coloration within the cytoplasm of epithelial cells was considered indicative of positive E-cadherin immunoreactivity [9].

Cytological Evaluation

Papanicolaou-stained smears were examined microscopically for cytopathological abnormalities, including infection, inflammation, keratinization, and cellular atypia. Additional features such as bi- or multinucleation and irregular nuclear morphology were recorded as indicators of 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 smokers (n=100) and non-smokers (n=100). The mean age of smokers was 28.4 ± 6.2 years, while that of non-smokers was 29.1 ± 5.8 years, with no statistically significant difference ($p = 0.42$). Most participants were between 26 and 35 years of age, and males constituted the majority in both groups (smokers: 85%, non-smokers: 82%; $p = 0.58$), as summarized in **Table 1**.

Table 1: Demographic Characteristics of Study Participants

Characteristic	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%)

Cytological examination revealed a significantly higher prevalence of abnormalities in smokers compared to non-smokers ($p < 0.05$). Inflammation, infection, cellular atypia, and keratinization were notably more common in the smoker group, as shown in Table 2.

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

Parameter	Smokers (n=100)	Non-Smokers (n=100)	p-value
Inflammation (%)	46 (46%)	18 (18%)	0.04
Infection (%)	12 (12%)	5 (5%)	0.03
Cellular atypia (%)	10 (10%)	2 (2%)	0.02
Keratinization (%)	8 (8%)	1 (1%)	0.01

Immunohistochemical analysis demonstrated a significantly higher rate of positive E-cadherin expression among smokers (23%) compared to non-smokers (3%) (**p** = 0.005). Conversely, negative expression was more common among non-smokers (97%) than smokers (73%), as detailed in **Table 3**.

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

Parameter	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%)	0.005

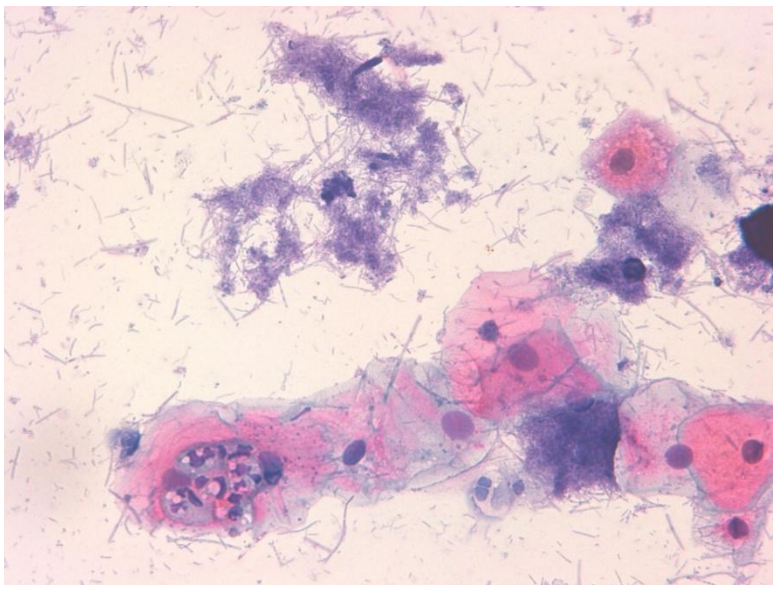

Figure 1: Microphotographs of Smear Samples from Buccal Mucosa Stained with Papanicolaou's Method (X40) Demonstrate Inflammatory Cells

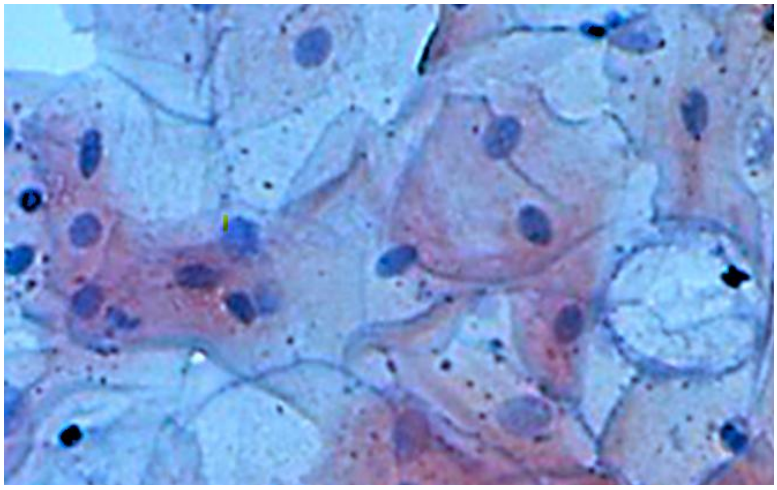

Figure 2: Microphotographs of buccal smears from smokers with immunohistochemical staining (x40) show the expression of E-cadherin in the brown color of the cytoplasm

4.Discussion

This study demonstrated significant cytological and molecular alterations in the oral mucosa of smokers compared with non-smokers, highlighting the harmful effects of tobacco exposure on epithelial cells. The higher rates of inflammation, infection, cellular atypia, and keratinization among smokers are consistent with earlier findings that chronic tobacco exposure induces epithelial injury and cellular degeneration [12,13]. These changes are indicative of early mucosal damage and may precede malignant transformation in long-term users.

E-cadherin, a key molecule responsible for cell-cell adhesion and epithelial integrity, showed a markedly altered expression pattern in smokers. Interestingly, smokers exhibited a higher proportion of positive E-cadherin staining compared to non-smokers. This pattern may reflect a compensatory cellular response to maintain epithelial cohesion under the stress of chronic tobacco exposure. Similar compensatory upregulation has been reported in early or pre-neoplastic lesions, suggesting a transient attempt to counteract cellular disruption before eventual downregulation in malignant progression [14,15].

These findings challenge the classical understanding that reduced E-cadherin expression universally accompanies carcinogenesis. Instead, early-phase alterations-either aberrant overexpression or loss of membranous localization-may occur in response to environmental stressors such as tobacco exposure [16,17]. The altered E-cadherin expression observed here could, therefore, represent an early biomarker of epithelial stress and an initial step in the carcinogenic cascade.

No significant differences in E-cadherin expression were found across age groups or between genders, confirming that tobacco exposure, rather than demographic variables, is the dominant factor influencing these molecular changes. Considering the high prevalence of smoking among young adults and males in Saudi Arabia, these findings underscore the urgent need for targeted prevention and awareness strategies to mitigate tobacco-related oral diseases [18].

5.Conclusions

This study confirms that smoking induces significant cytological abnormalities and alterations in E-cadherin expression in the oral mucosa, suggesting early epithelial disruption and increased susceptibility to carcinogenesis. The observed changes emphasize the biological impact of tobacco exposure even in clinically healthy individuals.

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