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Evaluation of Cytological Changes in Oral Mucosa among Smokers and e-cigarette Users

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Abstract

Objective: Oral cancer remains a major global health concern closely linked to tobacco use. With the increasing popularity of electronic cigarettes, it is essential to evaluate their biological impact on oral tissues. This study compared cytological changes in the oral mucosa of traditional cigarette smokers, e-cigarette users, and non-smokers in a Saudi population to understand potential precancerous risk.

Methods: Buccal mucosal samples were collected from 150 adults divided equally into three groups: traditional cigarette smokers, e-cigarette users, and non-smokers. The samples were stained using the Papanicolaou method and examined for pleomorphism, hyperchromatism, nuclear-to-cytoplasmic ratio, keratinization, inflammation, and infection. Statistical analysis was performed using chi-square and Fisher's exact tests, and the strength of associations was expressed as odds ratios with 95 percent confidence intervals.

Results: Significant cytological alterations were identified among smokers compared with non-smokers. Pleomorphism was observed in 83 percent of smokers and 6 percent of non-smokers (odds ratio 76.49, $P = 0.00000$). Hyperchromatism and an increased nuclear-to-cytoplasmic ratio were detected in 45 percent and 36 percent of smokers respectively, while absent in non-smokers ($P = 0.00000$ for both). Infection was significantly higher in smokers (52 percent) than in non-smokers (14 percent; odds ratio 6.65, $P = 0.00001$). Keratinization and inflammation were more frequent in smokers but without statistical significance. Comparison between traditional and e-cigarette users revealed no significant differences in any cytological parameter, indicating similar cellular effects.

Conclusion: Both traditional and e-cigarette smoking produce cytological alterations in the oral mucosa that may predispose to dysplastic transformation. E-cigarettes, despite being marketed as safer alternatives, demonstrated cytological patterns comparable to those of traditional smoking, highlighting the need for continued surveillance and public awareness.

Keywords: Vaping, electronic nicotine delivery systems, mouth neoplasms, tobacco use

Introduction

Oral cancer is considered a major health issue worldwide. In 2022, approximately 389,846 new cases and 188,438 cancer-related deaths were reported, highlighting the significant impact of oral cancer on global health.[1] The most common malignancy in the oral cavity is oral squamous cell carcinoma, accounting for approximately 95% of all malignant oral lesions.[2] Smoking serves as a significant etiological factor in the development of oral cancer, with a considerable risk of dysplastic lesions.[3]

Cigarette smoke comprises over 7,000 components, many of which are harmful to human health, with at least 69 identified as carcinogenic. Some of these substances, such as nicotine, occur naturally in tobacco, while others, such as ammonia, are introduced during manufacturing. Most harmful compounds, including acrolein, are generated when tobacco and paper are burned. The carcinogenic components found in cigarettes include benzene, 1,3-butadiene, and formaldehyde, as well as tobacco-specific nitrosamines, such as nicotine-derived nitrosamine ketone and *N*-nitrosonornicotine.[4]

The impact of smoking on oral health has been extensively documented in the literature. For example, Seifi et al.[5] found that smokers exhibited significant increases in nuclear size, the nuclear-to-cytoplasmic (N/C) ratio, and Feret ratio (F.R) in the lateral surface of the tongue, buccal mucosa, and floor of the mouth compared with non-smokers ($P \leq 0.001$). Additionally, smokers showed a higher prevalence of inflammation and *Candida* ($P < 0.001$).[5] Complementing these findings, Salih et al. used the Papanicolaou (Pap) staining technique to show that reactive changes were significantly more common in smokers (46.4%) compared to non-smokers (3.2%, $P = 0.001$). Among smokers, traditional cigarette users (51.4%) had a higher rate of reactive changes than electronic cigarette users (37.5%) and those who used both types (50.0%). Furthermore, flow cytometric analysis of oral keratinocytes from smokers revealed significant DNA damage, including increased DNA adducts and alterations in cell cycle dynamics, indicating an elevated risk of malignant transformation due to tobacco exposure.[6]

In recent years, e-cigarettes have become increasingly popular, especially among younger populations (18 years old and above), who often perceive them as safer and non-addictive alternatives to traditional cigarettes. These devices consist of a cartridge filled with flavoured e-liquid, a heating element to vaporize the e-liquid, and a rechargeable battery. These e-liquids often contain humectants, flavourings, and varying levels of nicotine, some even being nicotine-free. While using e-cigarettes (i.e. 'vaping') mimics the sensation of smoking without combustion-related harm, the heating process can produce potentially hazardous compounds.[7] When propylene glycol and glycerol are heated, they can produce thermal degradation products, including aldehydes such as formaldehyde and acetaldehyde, which are known carcinogens. These substances have been detected in emissions from various generations of e-cigarettes, with higher levels produced by newer models because of their increased heating power.[8–10]

While e-cigarette aerosols contain fewer carcinogens than traditional cigarette smoke, concerns remain about their potential to induce cancer. Since e-cigarettes have been in use for a relatively short time (since around 2007), the typical latency period for cancer may exceed the duration of e-cigarette use. The compounds present in e-cigarette aerosols, such as acrolein and formaldehyde, are known to have carcinogenic potential, but few oncology-related studies have been conducted using animals. In vitro studies have indicated that e-cigarette condensate can promote cell growth and metastasis in breast cancer cells.[11] Additionally, e-cigarette aerosols have been shown to induce DNA damage and hinder DNA repair in the lungs, bladder, and heart of mice.[12] In their significant study, Tang et al.[13] found that mice exposed to e-cigarette vapour developed lung adenocarcinomas and bladder hyperplasia over a year.

As the oral cavity is the first site of exposure during vaping, biological changes are expected to occur in oral tissues. Although limited data exist on the relationship between e-cigarette use and oral cancers, new evidence demonstrates that smoking e-cigarettes, whether with or without nicotine, is associated with increased oxidative stress and cellular damage, as indicated by elevated levels of malondialdehyde (MDA) and micronuclei (MN) scores. E-nicotine users also exhibit significantly higher salivary mucins compared to non-smokers, suggesting a more pronounced impact on oral health.[14] In a pilot study, Hamad et al.[15] demonstrated that buccal cells from e-cigarette users showed altered expression of tumour-suppressor and DNA repair genes after a 20-puff vaping session. Moreover, Pop et al.[3] found that e-cigarette use was associated with cytotoxic damage in the oral mucosa, as shown by the presence of micronuclei in oral epithelial cells. In addition, Salih et al.[16] demonstrated that e-cigarette users exhibited reactive changes in their buccal mucosa, with a prevalence of 37.5%. While these changes were less frequent than in traditional cigarette users (51.4%), they were still significant compared with non-smokers (3.2%). This indicates that e-cigarette use is associated with cytological alterations in oral tissues, underscoring the potential health risks linked to vaping.

While the detrimental effects of traditional smoking on oral mucosa have been well documented, the biological effects of vaping e-cigarettes are comparatively under-researched. Accordingly, the present study aimed to evaluate and compare the cytological changes in the oral mucosa of traditional cigarette smokers, e-cigarette users, and non-smokers in an adult Saudi population, focusing on cytological atypia, inflammatory responses, and infection rates to clarify the potential risks associated with different forms of smoking and to guide future preventive and clinical management strategies.

Beyond addressing this knowledge gap, the present study contributes novel evidence by providing the first cytological comparison between traditional and electronic cigarette users within a Saudi population. While several international studies have evaluated similar parameters, none have explored these cytological alterations in this regional context, where smoking and vaping behaviours are increasingly prevalent among younger adults. This investigation uniquely employs exfoliative cytology with Papanicolaou staining to assess a comprehensive spectrum of epithelial and nuclear changes, including pleomorphism, hyperchromatism, nuclear-to-cytoplasmic ratio, keratinization, inflammation, and microbial infection. By encompassing these parameters, the study establishes baseline cytological data for both traditional and e-cigarette users in Saudi Arabia and highlights their potential clinical implications for early detection and oral cancer prevention. The findings also challenge the common perception that e-cigarettes represent a harmless alternative to conventional tobacco, emphasizing that vaping may exert comparable cytological impacts on oral mucosa.

Materials and Methods

A cross-sectional analytical study was conducted to evaluate the cytological changes in oral mucosa among traditional cigarette smokers, e-cigarette users, and non-smokers. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of King Abdulaziz University, Rabigh, Saudi Arabia (KAU/FAMS-R/24-005; approval date: 18 Feb 2024). All participants provided written informed consent prior to enrolment

Participant Selection

A total of 150 healthy Saudi adults aged between 18 and 60 years were selected using convenience sampling and divided into three groups. Group A (i.e. traditional cigarette smokers) included individuals who reported smoking at least 10 conventional cigarettes daily for the past 12 months. Group B (i.e. e-cigarette users) included individuals who reported exclusive daily use of e-cigarettes for the past 12 months, with no concurrent use of traditional cigarettes. Finally, Group C (i.e. non-smokers) included individuals with no history of tobacco or e-cigarette use.

The exclusion criteria were the use of other tobacco products (e.g. cigars, pipes, chewing tobacco), chronic alcohol consumption, systemic diseases (e.g. diabetes, autoimmune disorders), ongoing orthodontic treatment, and any prior diagnosis of pre-malignant oral lesions.

Participants were recruited using convenience sampling, which may introduce an element of selection bias because recruitment depended on voluntary participation and self-reported smoking or vaping behavior. This approach was selected for practical and ethical reasons, as it enabled access to participants willing to disclose personal lifestyle habits within the study period. Nonetheless, the potential limitations of this sampling strategy are acknowledged, as it may not fully represent the characteristics of the broader Saudi population.

Sample Collection and Cytological Analysis

The participants were instructed to rinse their mouths with water prior to sample collection. To verify the adequacy of the sample size, a post-hoc power analysis was performed using G*Power version 3.1.9.7. Assuming a significance level (α) = 0.05, a statistical power of 0.80, and an expected medium effect size (Cohen's w = 0.3) for chi-square analysis, the minimum required sample size was estimated to be 108 participants. The inclusion of 150 participants in the present study therefore provided sufficient power to detect statistically meaningful intergroup differences in cytological alterations. Exfoliative cytology samples were obtained from the buccal mucosa using a sterile cytobrush. The collected cells were immediately smeared onto pre-labelled glass slides, fixed in 95% ethanol, and stained

using the Pap staining technique. Cytological evaluation was performed by two independent blinded cytopathologists who assessed the following parameters:

- Cellular atypia, including nuclear pleomorphism, hyperchromatism, increased N/C ratio, and the presence of bi- or multi-nucleated cells.
- Keratinization, that is, the presence of keratinized cells, which is indicative of epithelial maturation disturbances.
- Inflammation, characterized by the presence of inflammatory cells within the epithelial smear.
- The presence of microbial colonies or cytopathic effects suggestive of infection.

All cytological smears were independently examined by two qualified cytopathologists with over eight years of diagnostic experience. Both evaluators were blinded to the participants' group allocation to minimize assessment bias. Each smear was evaluated according to the established cytological criteria for pleomorphism, hyperchromatism, nuclear-to-cytoplasmic ratio, keratinization, inflammation, and infection. In cases of discrepancy, a joint review was conducted, and consensus was reached through discussion. Inter-rater reliability between the two cytopathologists was calculated using Cohen's kappa statistic ($\kappa = 0.87$), indicating excellent agreement.

Statistical Analysis

The data were analysed using SPSS software (v. 19.0; IBM SPSS, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were presented as frequencies and percentages. The chi-square test or Fisher's exact test was employed to assess associations between categorical variables. A *P*-value of ≤0.05 was considered statistically significant. Logistic regression analysis was considered but not applied, as the dataset did not include continuous or quantitative covariates such as age, duration and intensity of smoking or vaping, alcohol intake, or oral hygiene practices. Consequently, the use of non-parametric categorical tests was deemed most suitable for the available data. To improve the interpretability of results, odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for each cytological parameter, and exact *P*-values were reported instead of threshold values.

Results

This study enrolled 150 healthy Saudi adults (age range, 19–57 years; mean ± SD, 30.66 ± 10.33 years). The majority of participants were men (71%), while women accounted for 29%. The participants were equally distributed into three groups: Group A, traditional cigarette smokers ($n = 50$); Group B, e-cigarette users ($n = 50$); and Group C, control non-smokers ($n = 50$).

Cytological Alterations in Smokers Versus non-Smokers

The results of Pap-stained oral mucosal smears showed significant cytological differences between smokers and non-smokers. Pleomorphism was markedly more prevalent among smokers (83%) than non-smokers (6%) and demonstrated the strongest association with smoking (OR = 76.49, 95% CI 21.30–274.69, $P = 0.00000$) (Figures 1–4). Hyperchromatism,

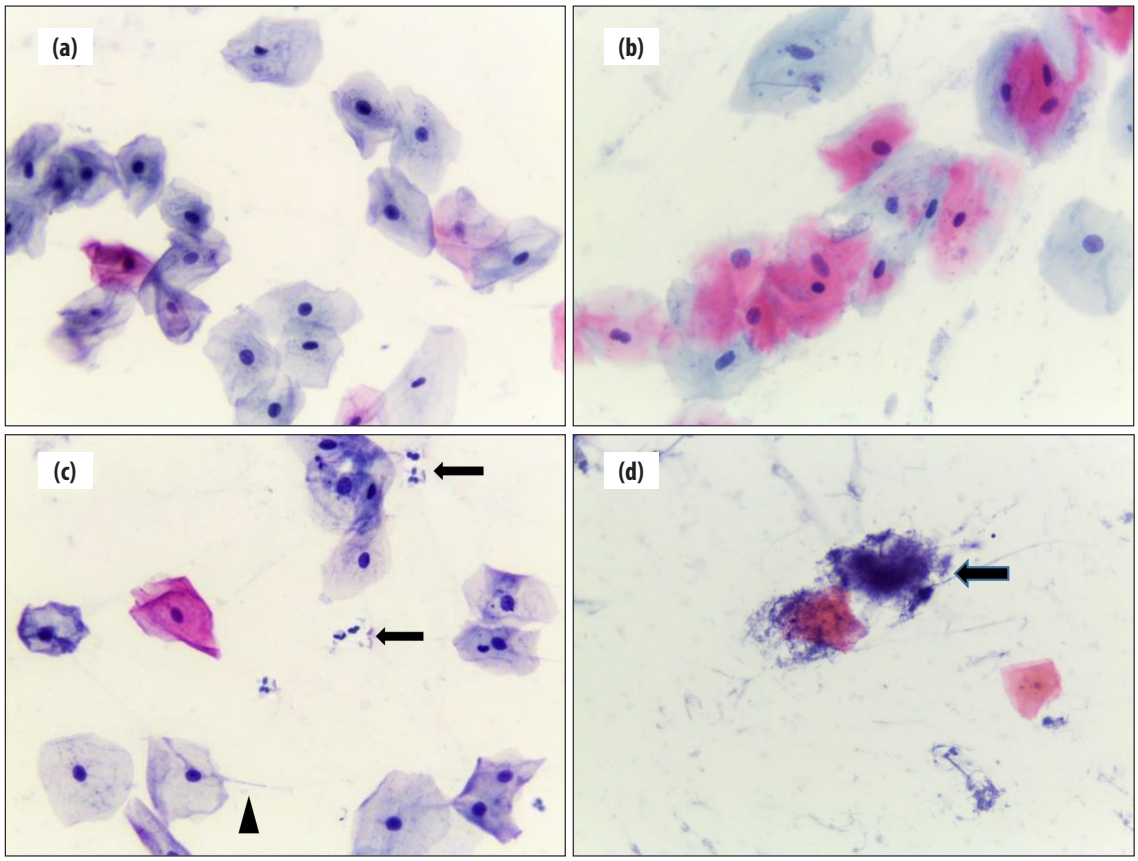

Fig. 1 **Representative oral mucosal cytology in non-smokers. (a, b) Normal cytology: flattened, polyhedral squamous epithelial cells, with more or less centrally located rounded or ovoid nuclei. (C) Inflammation with fine pretentious background (arrow head) & many neutrophils (arrows). (D) Infection (bacterial colonies -arrow). (Pap stain X400).**

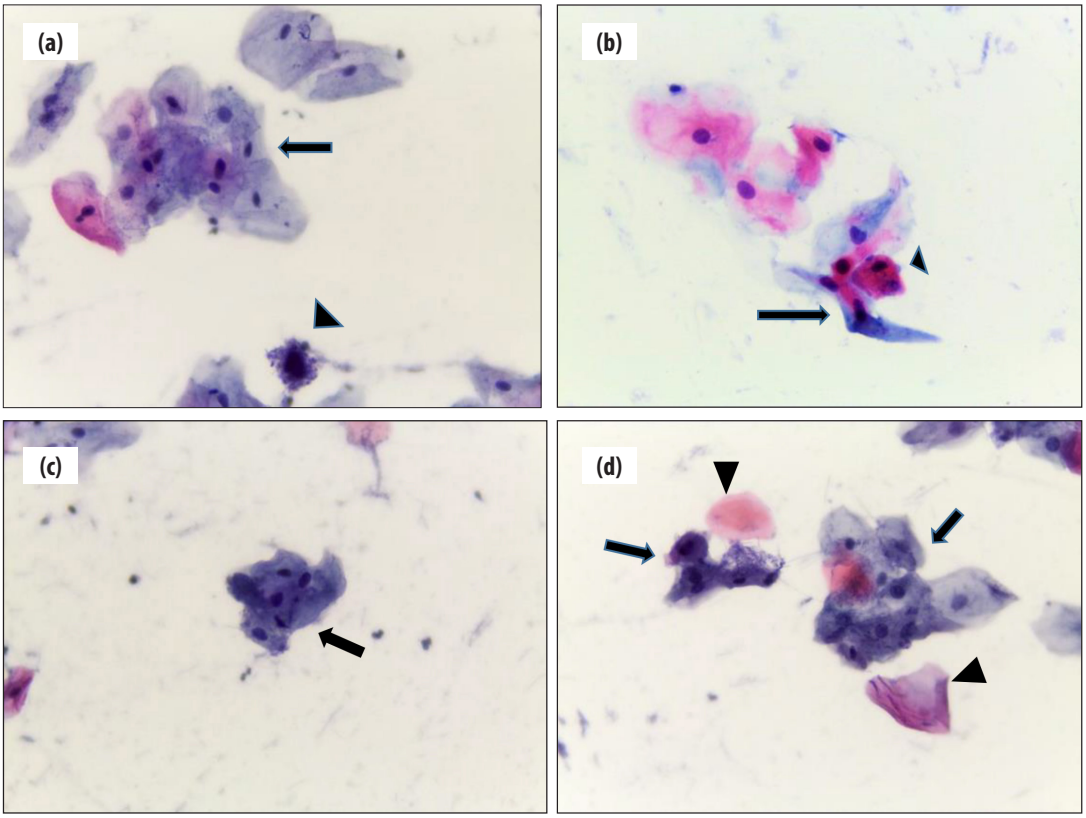

Fig. 2 **Cytological alterations in the buccal mucosa of traditional cigarette smokers. (a) Atypical cells with pleomorphic nuclei (arrow) and Infection (bacterial colonies- arrow head). (b) Pleomorphic cells with pleomorphic hyperchromatic nuclei (arrow) & cells with bi-nucleation (arrow head). (c) Group of atypical with increased N/C ratio (arrow). (d) Groups of atypical cells (arrows) & keratinization (arrow heads). (Pap stain X400).**

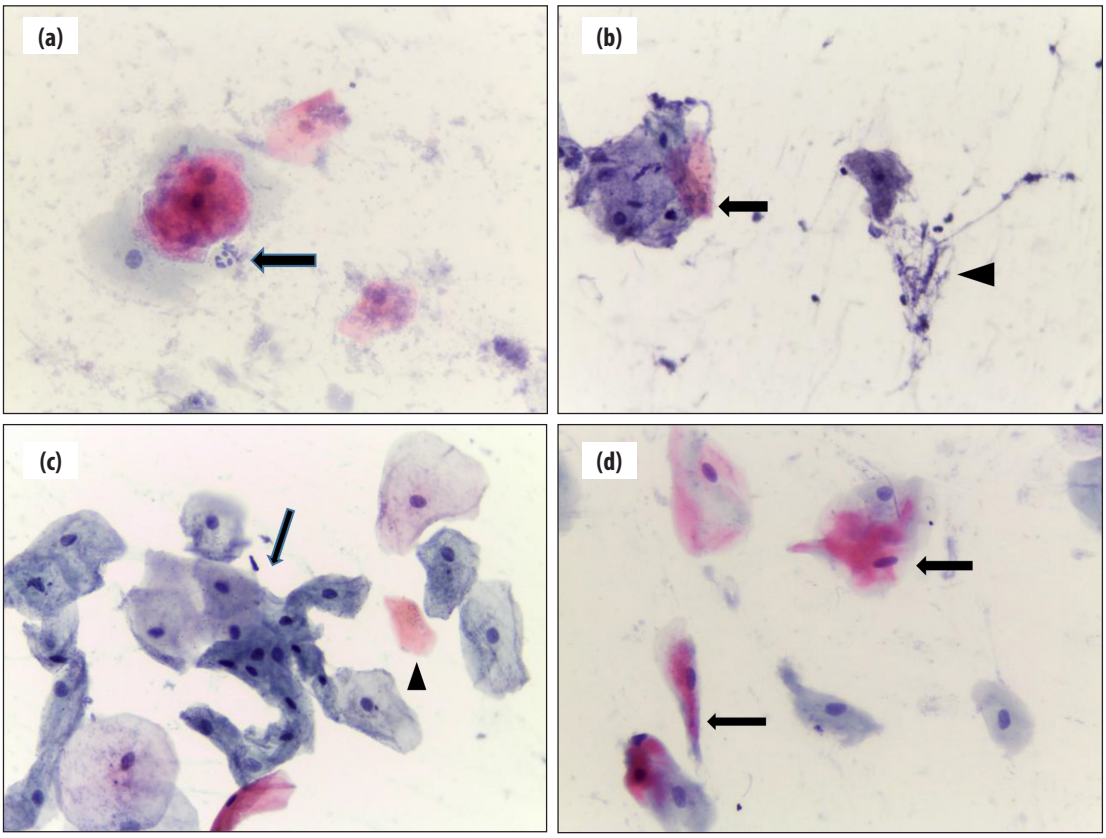

Fig. 3 **Cytological findings in e-cigarette users. (a) Inflammations (neutrophils- arrow) with dirty proteinaceous background. (b) Group of atypical cells with hyperchromatic nuclei (arrow), inflammatory cells entangled in fine proteinaceous threads (arrow head). (c) Group of atypical pleomorphic cells (arrow) & keratinization (arrow head). (d) Pleomorphic cells (arrow). (Pap stain X400).**

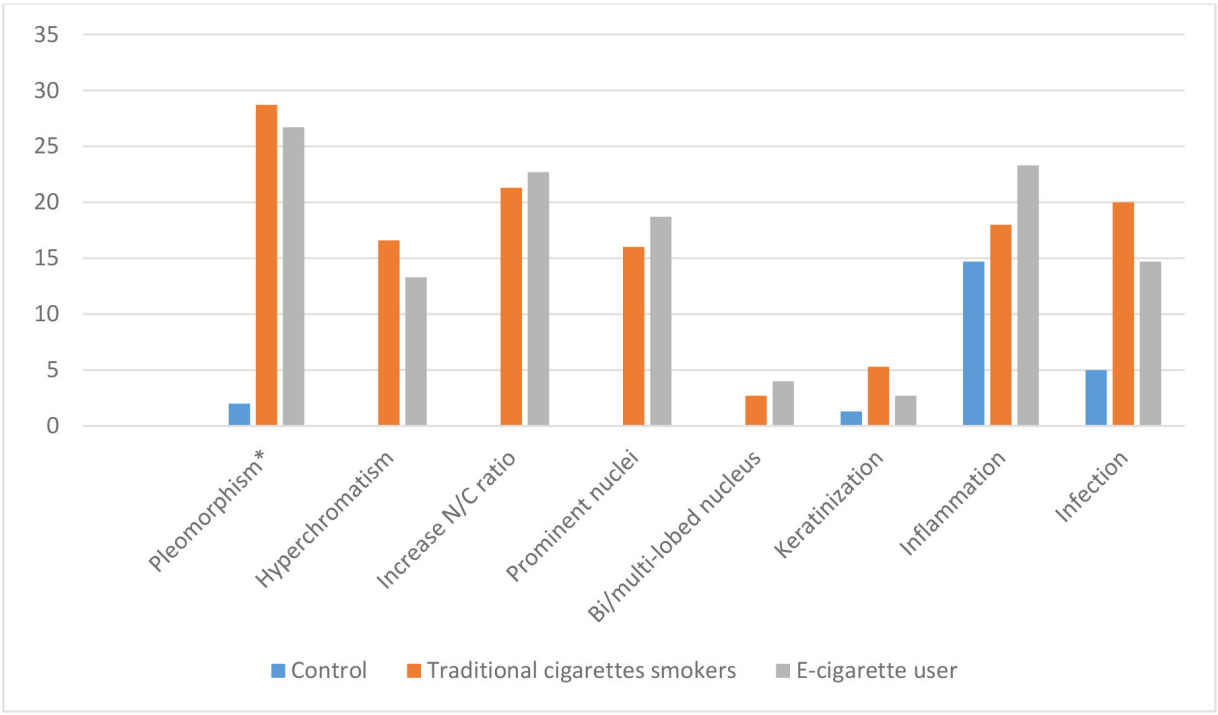

Fig. 4 **Prevalence of cytological abnormalities across study groups. Bar graph summarizing the frequency of pleomorphism, hyperchromatism, nuclear-to-cytoplasmic ratio increase, keratinization, inflammation, and infection among traditional cigarette smokers, e-cigarette users, and non-smokers.**

increased nuclear-to-cytoplasmic (N/C) ratio, and prominent nuclei were detected exclusively in smokers, producing extremely high odds ratios with significant *P*-values (all $P = 0.00000$). Bi- or multinucleated cells were observed in 10% of smokers and were absent in non-smokers (OR = ∞, 95% CI 0.64–194.25, $P = 0.02064$). Although keratinization (12% vs 4%; OR = 3.27, 95% CI 0.70–15.23, $P = 0.11234$) and inflammation (62% vs 46%; OR = 1.92, 95% CI 0.96–3.81, $P = 0.06230$) were more common among smokers, these differences were not statistically significant. Infection, however, was significantly higher among smokers (52%) compared with non-smokers (14%), with an odds ratio of 6.65 (95% CI 2.73–16.20, $P = 0.00001$). These findings highlight the strong relationship between smoking and cytological atypia of the oral mucosa, underscoring the heightened risk of epithelial dysplasia among smokers (Table 1).

Cytological Comparison Between Traditional Cigarette and e-Cigarette Users

Comparative analysis of oral mucosal cytology revealed minimal and statistically non-significant differences between traditional and e-cigarette users. Pleomorphism was slightly higher among traditional smokers (86%) than e-cigarette users (80%), but the difference was not significant (OR = 1.72, 95% CI 0.86–3.41, $P = 0.12136$). Similarly, hyperchromatism (50% vs 40%; OR = 1.45, 95% CI 0.82–2.57, $P = 0.19484$) and increased N/C ratio (64% vs 68%; OR = 1.14, 95% CI 0.64–2.05, $P = 0.65542$) showed no significant variation. The frequency of prominent nuclei was slightly higher in e-cigarette users (56%) than traditional smokers (48%) (OR = 1.44, 95% CI 0.82–2.51, $P = 0.20253$). Bi- or multinucleation (8% vs 12%; OR = 1.48, 95% CI 0.54–4.05, $P = 0.44686$) and keratinization (16% vs 8%; OR = 1.57, 95% CI 0.61–4.02, $P = 0.34578$) were also comparable between groups. Although inflammation appeared more prevalent among e-cigarette users (70%) than traditional smokers (54%), the difference was not significant (OR = 1.33, 95% CI 0.76–2.35, $P = 0.31510$). Likewise, infection was slightly higher in traditional smokers (60%) compared with e-cigarette users (44%) (OR = 1.32, 95% CI 0.76–2.31, $P = 0.32198$). These findings indicate that both smoking modalities exert a broadly comparable cytological impact on oral mucosa, with no statistically significant distinctions between the two groups (Table 2).

Discussion

This research strengthens the global evidence base by providing the first cytological comparison between traditional and e-cigarette users in a Saudi population, thereby addressing a regional data gap and contributing valuable insights relevant to oral health and cancer prevention awareness strategies in the Middle East. The foundations of exfoliative cytology trace back to the pioneering work of Papanicolaou, who first demonstrated the diagnostic value of epithelial smears for cancer detection.[17] His contributions established cytopathology as a cornerstone of early cancer screening and diagnostic research,[18,19] as later summarized comprehensively by Dugas et al.,[20] Incorporating these classical frameworks highlights the long-standing clinical relevance of cytological evaluation in detecting epithelial alterations caused by various environmental exposures, including smoking. Statistically significant cytological alterations were observed in the oral mucosa of both traditional cigarette smokers and e-cigarette users compared with non-smokers. The predominant findings included pleomorphism, hyperchromatism, an increased nuclear-to-cytoplasmic (N/C) ratio, and a higher frequency

Table 1. **The association of the results of Pap staining of oral mucosa cytology between control (non-smokers) and smokers**

Cytological feature	Non-smokers Present *n* (%)	Non-smokers Absent *n* (%)	Smokers Present *n* (%)	Smokers Absent *n* (%)	Odds Ratio (OR)	95% Confidence interval	Exact *P* value
Pleomorphism	3 (6%)	47 (94%)	83 (83%)	17 (17%)	76.49	21.30–274.69	0.00000
Hyperchromatism	0 (0%)	50 (100%)	45 (45%)	55 (55%)	∞	4.91–1363.56	0.00000
Increased N/C ratio	0 (0%)	50 (100%)	36 (36%)	64 (64%)	∞	3.37–939.37	0.00000
Prominent nuclei	0 (0%)	50 (100%)	52 (52%)	48 (48%)	∞	6.50–1805.03	0.00000
Bi or multilobed nucleus	0 (0%)	50 (100%)	10 (10%)	90 (90%)	∞	0.64–194.25	0.02064
Keratinization	2 (4%)	48 (96%)	12 (12%)	88 (88%)	3.27	0.70–15.23	0.11234
Inflammation	23 (46%)	27 (54%)	62 (62%)	38 (38%)	1.92	0.96–3.81	0.06230
Infection	7 (14%)	43 (86%)	52 (52%)	48 (48%)	6.65	2.73–16.20	0.00001

Table 2. **The association of the results of Pap staining of oral mucosa cytology between traditional cigarettes and e-cigarette smoking**

Cytological feature	Traditional Present *n* (%)	Traditional Absent *n* (%)	e-cigarette Present *n* (%)	e-cigarette Absent *n* (%)	Odds Ratio (OR)	95% Confidence interval	Exact *P* value
Pleomorphism	43 (86%)	7 (14%)	40 (80%)	10 (20%)	1.72	0.86–3.41	0.12136
Hyperchromatism	25 (50%)	25 (50%)	20 (40%)	30 (60%)	1.45	0.82–2.57	0.19484
Increased N/C ratio	32 (64%)	18 (36%)	34 (68%)	16 (32%)	1.14	0.64–2.05	0.65542
Prominent nuclei	24 (48%)	26 (52%)	28 (56%)	22 (44%)	1.44	0.82–2.51	0.20253
Bi or multilobed nucleus	4 (8%)	46 (92%)	6 (12%)	44 (88%)	1.48	0.54–4.05	0.44686
Keratinization	8 (16%)	42 (84%)	4 (8%)	46 (92%)	1.57	0.61–4.02	0.34578
Inflammation	27 (54%)	23 (46%)	35 (70%)	15 (30%)	1.33	0.76–2.35	0.31510
Infection	30 (60%)	20 (40%)	22 (44%)	28 (56%)	1.32	0.76–2.31	0.32198

of infection. These changes were observed in association with smoking behavior, as evidenced by markedly elevated odds ratios and highly substantial *P*-values (all $P \leq 0.0001$), corroborating previous findings.[3,5,21,22]

In agreement with Pop et al.[3] and Harrandah et al.,[23] our study confirmed notable cytological atypia in both e-cigarette users and traditional smokers. Pleomorphism was the most frequent atypical feature (83% of smokers vs 6% of non-smokers; OR = 76.49, 95% CI 21.30–274.69, $P = 0.00000$). Hyperchromatism, increased N/C ratio, and prominent nuclei were found exclusively among smokers, yielding extremely high odds ratios (all $P = 0.00000$). Bi- or multinucleation was observed in 10% of smokers but absent in controls (OR = ∞, $P = 0.02064$). Although keratinization (12% vs 4%; $P = 0.11234$) and inflammation (62% vs 46%; $P = 0.06230$) were more frequent in smokers, these differences did not reach statistical significance. Infection was substantially higher among smokers (52%) than non-smokers (14%) (OR = 6.65, 95% CI 2.73–16.20, $P = 0.00001$), supporting an association between smoking and mucosal microbial imbalance.

Keratinization and inflammation were elevated among smokers from both groups A and B, but only infection showed a noteworthy difference from controls. These observations align with Altom et al.,[21] who reported greater keratinization, inflammation, and infection among smokers, all reaching statistical significance. The noticeable rates of inflammation and keratinization in non-smokers (Group C) may reflect consumption of hot or spicy foods and mild alcohol intake, as suggested by Ahmed et al.,[24] who linked such dietary habits and alcohol exposure to epithelial keratinization.

Comparing traditional and e-cigarette users, our results revealed no statistically significant differences in any cytological parameter. Pleomorphism, hyperchromatism, infection, and inflammation occurred at comparable frequencies between groups (all $P > 0.05$), and the odds ratios were close to 1, indicating similar cytological risk profiles. Although traditional smokers exhibited marginally higher pleomorphism and infection rates, and e-cigarette users showed slightly greater inflammation, the overall cellular responses were largely equivalent. These findings support the observations of Harrandah et al.,[23] who noted the highest rates of cytological atypia among traditional cigarette users but comparable patterns between e-cigarette and hookah users. The increased prevalence of inflammatory changes among e-cigarette users in our cohort may be related to aerosol-induced oxidative stress and pro-inflammatory cytokine release. Pushalkar et al.[25] demonstrated that e-cigarette exposure disrupts the oral microbiome and elevates IL-6 and IL-1β levels, rendering users more susceptible to infection and mucosal dysregulation.

Conversely, other studies have reported that cytological alterations were limited to traditional smoking, with e-cigarette aerosols exerting minimal or no measurable impact on oral epithelium. Emma et al.[26] observed that cigarette smoke induced substantial cytotoxicity, mutagenicity, and genotoxicity, whereas e-cigarette aerosols caused negligible effects. Stewart et al.[27] similarly reported that tobacco smoking altered gut and oral microbiota, while e-cigarette use produced no notable microbial shifts.

In our study, the higher inflammatory rates among e-cigarette users may reflect the known cytotoxic potential of certain e-liquid constituents. Laboratory and animal studies have shown that e-cigarette aerosols can reduce cell proliferation and migration, enhance apoptosis, and increase oxidative

and inflammatory damage.[28–31] Although marketed as safer alternatives due to the absence of combustion-related carcinogens, accumulating evidence challenges this perception. Lee et al.[12] demonstrated that e-cigarette aerosol exposure induces mutagenic DNA adducts such as O^6-methyldeoxyguanosine and γ-hydroxy-1,N^2-propano-deoxyguanosine in vital organs, accompanied by down-regulation of DNA-repair proteins (XPC, OGG1/2). Such findings indicate potential carcinogenicity of vaping aerosols. Moreover, flavouring agents commonly used in e-liquids have been shown to possess independent cytotoxic and genotoxic properties,[32,33] further complicating the toxicological assessment of e-cigarette use.

Our study contributes unique region-specific data addressing the cytological impact of both traditional and e-cigarette smoking in Saudi Arabia, where the prevalence of tobacco use remains high and local evidence is scarce. The findings reinforce the growing consensus that e-cigarettes are not biologically inert and may exert cytological effects comparable to those of conventional smoking. Within the limitations of convenience sampling and absence of multivariate adjustment, the observed cytological abnormalities warrant further large-scale investigations incorporating quantitative exposure parameters and molecular endpoints to better understand the oncogenic potential of vaping and traditional smoking in this population.

While our findings demonstrate clear cytological differences between smokers, e-cigarette users, and non-smokers, these observations should be interpreted as associations rather than evidence of causation. The cross-sectional design precludes temporal or mechanistic inference. The mechanistic insights discussed, including oxidative stress, DNA damage, and microbiome disruption reported by Lee et al.[12] and Pushalkar et al.,[25] are derived from previous experimental and molecular studies and are presented here solely to contextualize our cytological observations.

Strengths and Limitations

The present study provides valuable cytological evidence comparing the oral mucosa of traditional cigarette smokers, e-cigarette users, and non-smokers in a Saudi population, thereby addressing an important regional gap in oral precancer screening data. Direct clinical assessment and cytological validation strengthen the reliability of the findings, while the inclusion of equal group sizes improves internal comparability. Although convenience sampling was used, a post-hoc power analysis confirmed that the inclusion of 150 participants exceeded the minimum required sample size to detect moderate effect differences between groups, ensuring adequate statistical power.

Despite these strengths, several limitations must be acknowledged. The cross-sectional design limits causal inference since the associations observed between smoking behaviors and cytological abnormalities represent correlations at a single point in time. The use of convenience sampling may also affect generalizability because participants who volunteered may differ in health awareness or smoking intensity from those who declined participation. Future studies that use randomized or stratified sampling strategies are recommended to minimize bias and improve external validity.

In addition, several potential confounding lifestyle factors, such as the duration and intensity of smoking or vaping, dietary patterns, alcohol consumption, and oral hygiene practices, were not controlled for in this study. These variables can influence epithelial regeneration, inflammatory responses, and microbial colonization of the oral mucosa, leading to inter-individual variation. The absence of multivariate statistical modeling is another limitation. Logistic regression could have provided adjusted estimates for potential confounders, but the dataset lacked detailed continuous or behavioral variables necessary for valid adjustment. Consequently, categorical tests such as the chi-square and Fisher's exact tests were applied. Nevertheless, the inclusion of odds ratios with 95 percent confidence intervals offers a meaningful estimation of association strength, partially compensating for the absence of multivariate analysis.

The study also lacked molecular and biochemical assays that could confirm the cytological findings at a mechanistic level. Techniques such as measurement of reactive oxygen species, detection of oxidative DNA adducts, or analysis of biomarker expression like p53 and Ki-67 would have strengthened biological interpretation. Previous studies by Lee et al.[12] and Pushalkar et al.[25] demonstrated that e-cigarette exposure can induce DNA damage, impair DNA repair activity, and disrupt the oral microbiome, supporting the need for molecular validation. Future research should integrate such endpoints and adopt longitudinal designs with multivariate control to clarify the temporal and mechanistic links between tobacco exposure and epithelial transformation.

Conclusion

Within the limitations of this cross-sectional study, both traditional cigarette smoking and e-cigarette use were associated with cytological alterations in the oral mucosa, including pleomorphism, hyperchromatism, and an increased nuclear-to-cytoplasmic ratio, when compared with non-smokers. These alterations may represent early epithelial stress responses rather than direct evidence of malignant transformation. No significant differences were observed between traditional and e-cigarette users, suggesting that e-cigarette aerosols can produce cytological patterns comparable to conventional tobacco smoke.

These results are consistent with those reported by Pop et al.[3] and Harrandah et al.,[23] who found similar epithelial atypia across smoking types, but differ from the findings of Emma et al.[26] and Stewart et al.,[27] who observed minimal cytotoxic effects from e-cigarette aerosols. This variation among studies highlights the complexity of exposure chemistry and underscores the importance of further controlled and mechanistic investigations.

In summary, the findings provide region-specific evidence that both traditional and electronic smoking behaviors adversely affect oral epithelial morphology. Although the present study does not establish a causal relationship between smoking and cancer development, it emphasizes the need for continued surveillance, public awareness, and preventive oral health programs. Larger prospective studies incorporating molecular biomarkers are necessary to determine the biological relevance and progression potential of these cytological changes.

Acknowledgment

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Disclosure Statement

In this manuscript, artificial intelligence (AI) tools were utilized to enhance the writing, improving clarity and readability.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of King Abdulaziz University, Rabigh, Saudi Arabia (approval code: KAU/FAMS-R/24-005; approval date: 18 Feb 2024). All participants provided written informed consent before participation.

Consent for Publication

Not applicable (no identifiable personal data or images are included). ■

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