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RESEARCH ARTICLE

COMPARATIVE CLINICAL EVALUATION AND HISTOPATHOLOGICAL ASSESSMENT OF ORAL MUCOSA IN CIGARETTE SMOKERS AND HEATED TOBACCO PRODUCT USERS

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Abstract

Background: Conventional cigarette smoking (CS) is a well-established risk factor for oral mucosal diseases, whereas the biological effects of heated tobacco products (HTPs) remain not fully understood. This study aimed to compare the clinical manifestations and histopathological characteristics of the oral mucosa in conventional CS and exclusive users of HTPs.

Methods: This cross-sectional comparative study included 83 tobacco users (47 conventional CS and 36 exclusive HTP users, all of whom had previously smoked conventional cigarettes) and 40 healthy nonsmoking controls. All participants underwent standardized oral clinical examination. For histopathological assessment, 20 participants were randomly selected from each tobacco-use group (20 CS and 20 HTP users), and incisional biopsies were obtained from oral mucosa in these participants. Histopathological evaluation was performed using hematoxylin-eosin and Van Gieson's picrofuchsin staining to assess epithelial architecture, inflammatory infiltration, vascular alterations, and connective tissue remodeling. Statistical analyses were conducted using IBM SPSS Statistics, with $p < 0.05$ considered statistically significant.

Results: Conventional CS exhibited significantly more frequent clinical oral lesions than HTP users, including diffuse erythema (21.3% vs. 5.6%), hyperkeratosis (12.8% vs. 0%), and leukoplakia (8.5% vs. 0%). Histopathological examination demonstrated significantly higher rates of epithelial hyperkeratosis (60.0% vs. 15.0%), acanthosis (55.0% vs. 10.0%), epithelial atrophy (35.0% vs. 5.0%), chronic inflammatory infiltration (80.0% vs. 30.0%), vascular congestion (75.0% vs. 20.0%), stromal edema (50.0% vs. 15.0%), collagen disorganization (65.0% vs. 10.0%), and early fibrosis (45.0% vs. 5.0%) in cigarette smokers compared with HTP users (all $p < 0.05$). Mild epithelial dysplasia was identified only among cigarette smokers (20.0%). Histological architecture remained largely preserved in HTP users, although mild inflammatory and connective tissue changes were occasionally observed.

Conclusions: Conventional CS was associated with substantially greater clinical and histopathological alterations to the oral mucosa than HTP use. Nevertheless, HTPs were also associated with measurable epithelial and stromal alterations, indicating that they are not biologically harmless. Regular oral examination of all tobacco users is warranted for the early detection of tobacco-associated mucosal lesions, while complete tobacco cessation remains the most effective strategy for preserving oral health.

Keywords: cleft lip; pediatric craniofacial surgery; cheiloplasty; multidisciplinary treatment; nasoalveolar molding; virtual surgical planning; three-dimensional imaging

1.INTRODUC TION

Smoking remains one of the leading preventable causes of morbidity and mortality worldwide and continues to represent a major public health challenge. In addition to its well-established association with cardiovascular disease, chronic respiratory disorders, and malignancies, tobacco use exerts profound adverse effects on the oral cavity. Cigarette smoking (CS) is recognized as an important etiological factor in the development of oral mucosal lesions, periodontal disease, delayed wound healing, and oral squamous cell carcinoma through chronic exposure to carcinogens, oxidative stress, and persistent inflammation[1-3].

The oral mucosa is among the first tissues directly exposed to tobacco smoke and therefore undergoes continuous physical and chemical irritation[2]. Long-term smoking induces a broad spectrum of clinical and microscopic alterations, including hyperkeratosis, epithelial hyperplasia, acanthosis, epithelial dysplasia, vascular congestion, inflammatory cell infiltration, and connective tissue remodeling. These histopathological changes may precede clinically detectable lesions and contribute to the development of potentially malignant disorders[1, 2, 16, 17].

In recent years, heated tobacco products (HTPs) have gained worldwide popularity as an alternative to conventional cigarettes. Unlike combustible cigarettes, HTPs heat processed tobacco without combustion, thereby reducing the formation of numerous toxic combustion products. Nevertheless, HTP aerosols still contain nicotine together with some potentially harmful chemicals capable of affecting oral tissues. Current evidence suggests that although HTPs may reduce exposure to some toxicants compared with conventional cigarettes, they cannot be considered harmless, and their long-term biological effects remain insufficiently characterized[5, 6, 20, 23].

Recent clinical investigations have reported differences in oral health status between cigarette smokers and HTP users. Conventional smokers generally demonstrate a higher prevalence of smoker's melanosis, nicotine stomatitis, periodontal inflammation, and oral mucosal lesions, whereas HTP users may exhibit milder clinical manifestations. However, microscopic alterations have also been described in users of alternative nicotine products, including epithelial hyperparakeratosis, acanthosis, chronic inflammatory infiltration, and stromal edema, indicating that these products are not biologically inert[4, 7-10].

Despite the growing number of studies evaluating the clinical effects of HTPs, direct comparative investigations of both the clinical manifestations and histopathological characteristics of the oral mucosa in CS and HTP users remain limited[3, 7-9]. Histopathological assessment provides objective evidence of tissue alterations and allows the identification of early epithelial, vascular, and inflammatory alterations that may not yet be clinically apparent[1, 11]. Therefore, comparative clinicopathological evaluation is essential for improving our understanding of the biological effects of different tobacco products on the oral mucosa and for guiding preventive and therapeutic strategies in dental practice.

The aim of the study was to compare the clinical manifestations and histopathological features of the oral mucosa in cigarette smokers and users of heated tobacco products.

MATERIAL AND METHODS

Study design and participants

This cross-sectional comparative study was conducted at the Scientific Educational Dental Center No. 1 of Yerevan State Medical University, Yerevan, Armenia, from November 2025 to August 2026.

A total of 83 participants aged 19–62 years were enrolled in the study. The study population consisted of 47 conventional cigarette smokers (CS) and 36 users of heated tobacco products (HTPs).

Participants were classified according to their current tobacco-use pattern. The conventional cigarette smoking (CS) group consisted of current cigarette smokers, whereas the HTP group consisted of participants who were currently using heated tobacco products exclusively and had completely switched from conventional cigarettes to HTPs (reported duration of exclusive HTP use ranging from 5 to 8 years before enrollment). Importantly, all participants in the HTP group had a previous history of conventional cigarette smoking, having smoked conventional cigarettes for approximately 4–6 years before switching to HTPs. The reported duration of current conventional cigarette smoking among CS participants ranged from 9 to 14 years.

Thus, the HTP group represented former conventional cigarette smokers who had transitioned to exclusive HTP use. The study group included 45 men (54.2%) and 38 women (45.8%), with a mean age of 35.0 ± 15.6 years (mean ± SD).

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The control group comprised 40 healthy volunteers who had never smoked cigarettes or used HTPs. Their ages ranged from 20 to 68 years and included 23 men (57.5%) and 17 women (42.5%), with a mean age of 42.4 ± 15.5 years (mean ± SD).

All participants provided written informed consent prior to enrollment in the study.

Clinical examination

All participants underwent a comprehensive oral examination performed by the same investigator under standardized clinical conditions. Clinical assessment included a systematic examination of the lips, buccal mucosa, tongue, floor of the mouth, hard and soft palate, and gingival tissues[12].

Particular attention was paid to the color, surface texture, moisture, and consistency of the oral mucosa, as well as to the presence or absence of primary and secondary oral lesions. Clinical findings, including hyperkeratosis, leukoplakia, smoker's melanosis, erythema, erosions, ulcerations, traumatic lesions, and inflammatory changes, were recorded according to internationally accepted diagnostic criteria[11-13]

Histopathological examination

For histopathological assessment, 20 participants were randomly selected from each tobacco-use group (20 conventional cigarette smokers and 20 exclusive HTP users). All selected participants underwent incisional biopsy of oral mucosa. Thus, the histopathological analysis was performed on a randomly selected subset of participants from each tobacco-use group and represents the characteristics of both clinically identified oral mucosal lesions, as well as from clinically normal oral mucosa in these participants. The findings should not be interpreted as a histological assessment of the entire oral mucosa of all study participants.

For histological reference, one archival specimen of clinically normal oral mucosa from a nonsmoking individual was examined. This specimen was used solely as a morphological reference for normal oral mucosal architecture and was not considered a participant-level histological control group. The clinical control group consisted of 40 healthy nonsmoking volunteers; however, histopathological biopsies were not obtained from these individuals. The corresponding image is shown in Figure 1. Tissue samples were fixed in 10% neutral buffered formalin, dehydrated through graded alcohols, embedded in paraffin, and sectioned at a thickness of 4 μm. Sections were routinely stained with hematoxylin-eosin for the evaluation of general tissue architecture, epithelial morphology, inflammatory cell infiltration, vascular alterations, and degenerative changes[14]. Additional sections were stained with Van Gieson's picrofuchsin stain to differentiate collagen fibers from other connective tissue elements and to assess connective tissue organization, stromal remodeling, and the degree of fibrosis. Histological examination was performed using a ZEISS Primo Star trinocular microscope (ZEISS Microscopy, Germany) at ×100 and ×400 magnifications. Digital microphotographs were obtained using a ZEISS Axiocam ERc 5s camera system (Carl ZEISS Microscopy, Germany). Histopathological evaluation was performed according to internationally accepted histological criteria and the recommendations of the WHO[11].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± standard deviation (SD), whereas categorical variables are expressed as frequencies and percentages. Comparisons between groups were performed using the Student's t-test or the Mann–Whitney U test, depending on data distribution. Categorical variables were analyzed using the chi-square (χ^2) test or Fisher's exact test, as appropriate. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Ethical Considerations

This work was approved by the Ethical Committee at Yerevan State Medical University, done in compliance with the Helsinki Declaration, and written informed consent was obtained from the patients.

MATERIALS AND METHODS

Study design and setting

This cross-sectional comparative study was conducted at the Scientific Educational Dental Center No. 1 of Yerevan State Medical University, Yerevan, Armenia, between November 2025 and August 2026. The study was designed to compare clinical manifestations and histopathological characteristics of the oral mucosa among conventional cigarette smokers, exclusive heated tobacco product (HTP) users, and healthy nonsmoking controls.

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Study participants

A total of 123 participants were included in the clinical component of the study: 47 conventional cigarette smokers (CS), 36 exclusive HTP users, and 40 healthy nonsmoking controls. Participants were 19–68 years of age.

The conventional cigarette smoking group comprised current cigarette smokers. The HTP group comprised participants who were currently using HTPs exclusively and who had previously smoked conventional cigarettes. According to the reported tobacco-use history, exclusive HTP use had continued for approximately 5–8 years before enrollment, whereas previous conventional cigarette smoking among HTP users had lasted approximately 4–6 years. Participants in the conventional cigarette smoking group reported current cigarette smoking for approximately 9–14 years.

Thus, the HTP group represented former conventional cigarette smokers who had subsequently transitioned to exclusive HTP use rather than tobacco-naïve HTP users. This distinction was considered when interpreting between-group differences.

The study population included 45 men (54.2%) and 38 women (45.8%) among the tobacco-use groups, with a mean age of 35.0 ± 15.6 years. The healthy control group consisted of 40 volunteers who reported never having smoked conventional cigarettes or used HTPs. The control group included 23 men (57.5%) and 17 women (42.5%), with a mean age of 42.4 ± 15.5 years.

All participants provided written informed consent before enrollment in the study.

Clinical oral examination

All participants underwent a comprehensive clinical examination of the oral cavity performed by the same investigator under standardized clinical conditions. The examination included systematic assessment of the lips and oral commissures, buccal mucosa, tongue, floor of the mouth, hard and soft palate, and gingival tissues.

The oral mucosa was assessed with particular attention to color, surface characteristics, moisture, consistency, and the presence or absence of clinically detectable pathological changes.

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Clinical findings recorded during the examination included hyperkeratotic lesions, leukoplakia, smoker's melanosis, diffuse erythema, erosions, ulcerations, traumatic lesions, mucosal edema, xerostomia-related findings, and inflammatory changes.

Gingival bleeding on probing was also recorded as part of the clinical oral examination. Clinical findings were documented according to established clinical diagnostic principles and relevant internationally accepted criteria for oral mucosal lesions. Because the present study was observational and cross-sectional, the clinical findings were interpreted as observed associations rather than evidence of causality.

Histopathological assessment

Histopathological assessment was performed in a subset of participants from the two tobacco-use groups. Twenty participants were selected from the conventional cigarette smoking group and 20 from the exclusive HTP group, resulting in a total histopathological sample of 40 tobacco users.

The selected participants underwent incisional biopsy of oral mucosal tissue. The histopathological subset therefore represented a sample of the clinical study population rather than the entire cohort. The biopsy specimens included tissue obtained from clinically identified oral mucosal lesions as well as clinically normal-appearing oral mucosa in selected participants. Accordingly, the histopathological findings should not be interpreted as representing the histological status of the entire oral mucosa of all participants.

For morphological reference, one archival specimen of clinically normal oral mucosa obtained from a nonsmoking individual was examined. This archival specimen was used exclusively as a morphological reference for normal oral mucosal architecture and was not considered a participant-level histopathological control group. No inferential statistical comparison was performed between the archival specimen and the participant groups.

The clinical control group consisted of 40 healthy nonsmoking volunteers; however, histopathological biopsies were not obtained from these individuals. Consequently, the nonsmoking control group served as a clinical control group, whereas the archival specimen served only as a morphological histological reference. Tissue samples were fixed in 10% neutral buffered formalin, processed through graded concentrations of alcohol for dehydration, embedded in paraffin, and sectioned at a thickness of 4 μm.

Sections were routinely stained with hematoxylin and eosin (H&E) for assessment of overall tissue architecture, epithelial morphology, inflammatory-cell infiltration, vascular alterations, and degenerative or structural changes. Additional sections were stained with Van Gieson's picrofuchsin stain to facilitate differentiation of collagen fibers from other connective-tissue elements and to assess connective-tissue organization, stromal remodeling, and fibrotic changes.

Histological examination was performed using a ZEISS Primo Star trinocular microscope (ZEISS Microscopy, Germany) at ×100 and ×400 magnifications. Digital microphotographs were obtained using a ZEISS Axiocam ERc 5s camera system (Carl ZEISS Microscopy, Germany).

The histopathological parameters evaluated included epithelial hyperkeratosis, epithelial acanthosis, epithelial atrophy, chronic inflammatory-cell infiltration, vascular congestion, stromal edema, collagen-fiber disorganization, early fibrosis, and epithelial dysplasia.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Continuous variables were summarized using the mean and standard deviation (SD), while categorical variables were summarized using absolute frequencies and percentages.

For continuous variables, assessment of distributional assumptions should precede selection of the statistical test. Normally distributed continuous variables were compared using the Student's *t*-test, whereas non-normally distributed variables were compared using the Mann–Whitney U test, as appropriate.

Categorical variables were compared using Pearson's chi-square test when the expected cell frequencies were adequate. For sparse contingency tables, Fisher's exact test was used. Because several clinical outcomes contained small expected frequencies, the choice of the exact test should be explicitly reported in the final statistical output for each affected comparison.

For the histopathological comparison between the conventional cigarette smoking and exclusive HTP groups, Fisher's exact test is preferred because of the small sample size (20 participants per group) and the presence of low expected cell frequencies.

All statistical tests were two-tailed. A nominal p value <0.05 was considered statistically significant. Because multiple histopathological endpoints were evaluated, the final analysis should additionally report an appropriate multiplicity-adjusted significance assessment, preferably using the Holm procedure, or explicitly identify the histopathological analysis as exploratory if no multiplicity adjustment was performed.

Where feasible, effect estimates with 95% confidence intervals should be reported in addition to p values to provide information about the magnitude and precision of between-group differences.

No causal inference was intended because of the cross-sectional study design.

Ethical considerations

The study was approved by the Ethics Committee of Yerevan State Medical University and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment.

RESULTS

Clinical characteristics and findings of the oral mucosa

A total of 123 participants were included in the clinical analysis: 40 healthy nonsmoking controls, 36 exclusive heated tobacco product (HTP) users, and 47 conventional cigarette smokers (CS). All participants underwent a standardized clinical examination of the oral cavity, including assessment of the lips and oral commissures, buccal mucosa, tongue, floor of the mouth, palate, and gingival tissues.Participants reported a range of subjective oral symptoms, including oral discomfort, xerostomia, lip tightness, soreness at the oral commissures, burning or tingling sensations of the tongue, taste alterations, tongue coating, and a rough sensation of the oral mucosa. These complaints were recorded descriptively during the clinical examination and were not incorporated into a composite symptom score.

The distribution of clinically detected oral mucosal findings across the three study groups is presented in **Table 1**.

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Table 1. Clinical findings of the oral mucosa in cigarette smokers (CS), heated tobacco product (HTP) users, and healthy controls

Clinical finding	Control (n=40) n (%)	HTPs (n=36) n (%)	CS (n=47) n (%)	p value**
Normal oral mucosa*	36 (90.0)	28 (77.8)	9 (19.1)	**<0.001**
Diffuse mucosal erythema	1 (2.5)	2 (5.6)	10 (21.3)	**0.009**
Hyperkeratotic lesions	0 (0.0)	0 (0.0)	6 (12.8)	**0.006**
Smoker's melanosis	0 (0.0)	0 (0.0)	3 (6.4)	0.083
Leukoplakia	0 (0.0)	0 (0.0)	4 (8.5)	**0.035**
Gingival bleeding on probing	1 (2.5)	3 (8.3)	7 (14.9)	0.129
Xerostomia	1 (2.5)	1 (2.8)	3 (6.4)	0.591
Mucosal edema	1 (2.5)	2 (5.6)	5 (10.6)	0.097

Notes: Values are presented as *n (%)*.

- p values represent the overall comparison among the three study groups and were calculated using the Pearson χ^2 test.
  † Normal oral mucosa indicates absence of clinically detectable pathological alterations at the time of examination.

Abbreviations: CS, conventional cigarette smokers; HTP, heated tobacco product users; CI, confidence interval.

Overall p values represent the three-group comparison. Categorical comparisons were performed using Pearson's χ^2 test or Fisher's exact test, as appropriate. Cramer's V is reported as a measure of overall association. Risk differences represent CS prevalence minus HTP prevalence and are presented with approximate 95% confidence intervals. Pairwise CS-versus-HTP comparisons were performed using Fisher's exact test when sparse cell counts were present. No correction for multiple comparisons was applied.

*Normal oral mucosa was defined as the absence of clinically visible pathological changes, including inflammatory, keratotic, pigmentary, erosive, ulcerative, edematous, or clinically detectable gingival abnormalities.

Clinical findings

Clinically normal oral mucosa was observed in 36 of 40 healthy controls (90.0%), 28 of 36 HTP users (77.8%), and 9 of 47 CS participants (19.1%). The overall distribution differed significantly among the three groups ($p < 0.001$; Cramer's V = 0.651). Compared with HTP users, CS participants had a 58.6-percentage-point lower prevalence of clinically normal oral mucosa (95% CI, −76.3 to −41.0; $p < 0.001$).

Diffuse mucosal erythema was identified in 1 control participant (2.5%), 2 HTP users (5.6%), and 10 CS participants (21.3%). The overall three-group comparison was statistically significant ($p = 0.009$; Cramer's V = 0.277). The prevalence was 15.7 percentage points higher among CS participants than among HTP users (95% CI, 1.8 to 29.6); however, the direct CS-versus-HTP comparison did not reach conventional statistical significance ($p = 0.060$). Thus, the overall three-group difference should not be interpreted as definitive evidence of a statistically significant difference specifically between CS and HTP users. Hyperkeratotic lesions were detected in 6 CS participants (12.8%) and in none of the HTP users or controls. The overall association was statistically significant ($p = 0.006$; Cramer's V = 0.288). The prevalence difference between CS and HTP users was 12.8 percentage points (95% CI, 3.2 to 22.3; $p = 0.034$).

Smoker's melanosis was observed in 3 CS participants (6.4%) but in none of the HTP users or controls. The overall comparison did not reach statistical significance ($p = 0.083$; Cramer's V = 0.201), and the direct CS-versus-HTP comparison was also not statistically significant ($p = 0.254$). Leukoplakia was observed exclusively in the CS group, occurring in 4 of 47 participants (8.5%). No cases were identified among HTP users or controls. Although the overall three-group comparison reached statistical significance ($p = 0.035$; Cramer's V = 0.233), the direct CS-versus-HTP comparison did not reach statistical significance by Fisher's exact test ($p = 0.129$). The observed prevalence difference was 8.5 percentage points (95% CI, 0.5 to 16.5). Because of the small number of events, this finding should be interpreted cautiously.

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Gingival bleeding on probing was recorded in 1 control participant (2.5%), 3 HTP users (8.3%), and 7 CS participants (14.9%). The overall difference was not statistically significant (p = 0.129; Cramer's V = 0.183). The prevalence difference between CS and HTP users was 6.6 percentage points (95% CI, −7.0 to 20.2; p = 0.502).

Xerostomia was reported in 1 control participant (2.5%), 1 HTP user (2.8%), and 3 CS participants (6.4%). No significant overall association was observed (p = 0.591; Cramer's V = 0.092). The CS-versus-HTP prevalence difference was 3.6 percentage points (95% CI, −5.2 to 12.4; p = 0.629).

Mucosal edema was present in 1 control participant (2.5%), 2 HTP users (5.6%), and 5 CS participants (10.6%). The overall comparison was not statistically significant (p = 0.297; Cramer's V = 0.141), and the direct CS-versus-HTP comparison was also not significant (p = 0.693).

Overall, conventional cigarette smokers had the lowest prevalence of clinically normal oral mucosa and the highest observed prevalence of several pathological findings. HTP users showed an intermediate clinical profile between conventional cigarette smokers and healthy controls. These findings demonstrate an association between current tobacco-use category and clinical oral mucosal status but do not establish causality.

Histopathological findings

Histopathological examination was performed in a randomly selected subset of 40 tobacco users, comprising 20 conventional cigarette smokers and 20 exclusive HTP users. The histopathological analysis therefore represents a participant-level subset and should not be interpreted as a histological assessment of all 123 clinically examined participants. An archival specimen of clinically normal oral mucosa from a nonsmoking individual was examined solely as a morphological reference. Because this specimen did not represent a participant-level control group, it was not included in inferential statistical analyses.

The distribution of predefined histopathological findings among HTP users and CS participants is presented in **Table 2**.

Table 2 Semiquantitative histopathological evaluation of oral mucosal alterations in cigarette (CS) smokers, and heated tobacco product (HTP) users

Pathomorphological signs / Histopathological finding	HTPs (n=20) n (%)	CS (n=20) n (%)	p value*
Mild hyperkeratosis	3 (15.0)	12 (60.0)	**0.008**
Epithelial acanthosis	2 (10.0)	11 (55.0)	**0.006**
Epithelial atrophy	1 (5.0)	7 (35.0)	**0.044**
Chronic inflammatory infiltration	6 (30.0)	16 (80.0)	**0.004**
Vascular congestion	4 (20.0)	15 (75.0)	**<0.001**
Stromal edema	3 (15.0)	10 (50.0)	**0.036**
Collagen fiber disorganization	2 (10.0)	13 (65.0)	**<0.001**
Early fibrosis	1 (5.0)	9 (45.0)	**0.006**
Mild epithelial dysplasia	0 (0.0)	4 (20.0)	**0.035**

***** Data are presented as *n* (%). Between-group comparisons were performed using the two-sided Fisher's exact test because of the small sample size and low expected cell frequencies. A *P* value <0.05 was considered statistically significant.

Abbreviations: CS, conventional cigarette smokers; HTP, heated tobacco product users; CI, confidence interval.

Data are presented as n (%). Because of the small sample size and sparse cell counts, two-sided Fisher's exact tests were used for group comparisons. Risk differences represent CS prevalence minus HTP prevalence and are presented with approximate 95% confidence intervals. The archival nonsmoking specimen was not included in statistical comparisons.

An archival specimen of clinically normal oral mucosa demonstrated preserved stratified squamous epithelial
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architecture, regular epithelial maturation, an intact epithelial-connective tissue interface, and relatively uniform collagen organization with only occasional inflammatory cells (**Figure 1**). This specimen served exclusively as a morphological reference and was not used as a statistical control.

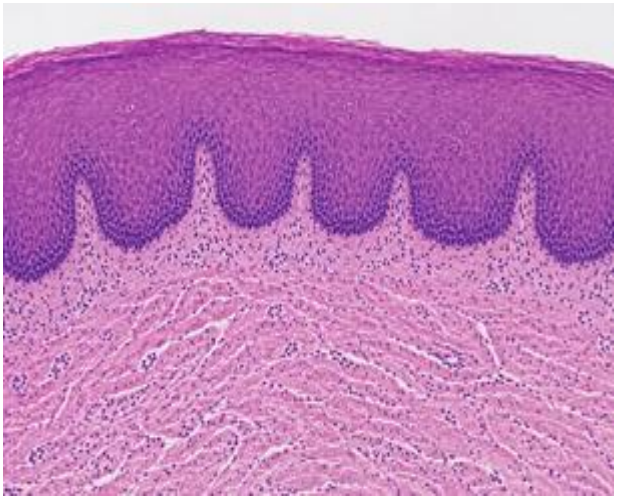

Figure 1. Representative histological appearance of clinically normal oral mucosa from an archival nonsmoking specimen used as a morphological reference.

Hematoxylin and eosin staining, ×100. The specimen demonstrates normally stratified squamous epithelium with preserved maturation and thickness, an intact epithelial-connective tissue interface, and no substantial inflammatory or stromal abnormalities.

In conventional cigarette smokers, hyperkeratosis was identified in 12 of 20 specimens (60.0%), compared with 3 of 20 HTP specimens (15.0%). The absolute prevalence difference was 45.0 percentage points (95% CI, 18.4 to 71.6; p = 0.008). Epithelial acanthosis was identified in 11 CS specimens (55.0%) compared with 2 HTP specimens (10.0%), corresponding to a 45.0-percentage-point difference (95% CI, 19.5 to 70.5; p = 0.006). Epithelial atrophy was present in 7 CS specimens (35.0%) and 1 HTP specimen (5.0%), with an absolute difference of 30.0 percentage points (95% CI, 7.0 to 53.0; p = 0.044).

The CS specimens demonstrated prominent inflammatory and vascular alterations. Chronic inflammatory infiltration was observed in 16 of 20 CS specimens (80.0%) compared with 6 of 20 HTP specimens (30.0%), yielding an absolute prevalence difference of 50.0 percentage points (95% CI, 23.3 to 76.7; p = 0.004). The inflammatory infiltrate was predominantly lymphoplasmacytic in morphology.

Vascular congestion was identified in 15 CS specimens (75.0%) compared with 4 HTP specimens (20.0%), corresponding to an absolute difference of 55.0 percentage points (95% CI, 29.2 to 80.8; p = 0.001). Stromal edema was observed in 10 CS specimens (50.0%) and 3 HTP specimens (15.0%), representing an absolute difference of 35.0 percentage points (95% CI, 8.1 to 61.9; p = 0.041).

Van Gieson's picrofuchsin staining demonstrated greater connective tissue remodeling in the CS group (**Figures 2C and 2D**). Collagen fiber disorganization and fragmentation were identified in 13 CS specimens (65.0%) compared with 2 HTP specimens (10.0%), corresponding to an absolute prevalence difference of 55.0 percentage points (95% CI, 30.3 to 79.7; p < 0.001). Early fibrotic changes with increased collagen deposition were present in 9 CS specimens (45.0%) and 1 HTP specimen (5.0%), yielding an absolute difference of 40.0 percentage points (95% CI, 16.2 to 63.8; p = 0.008).

Representative histopathological findings in conventional cigarette smokers are shown in **Figure 2**. Hematoxylin and eosin staining demonstrated epithelial hyperkeratosis, acanthosis, inflammatory infiltration, vascular congestion, and stromal changes, whereas Van Gieson's staining demonstrated collagen disorganization and increased collagen deposition.

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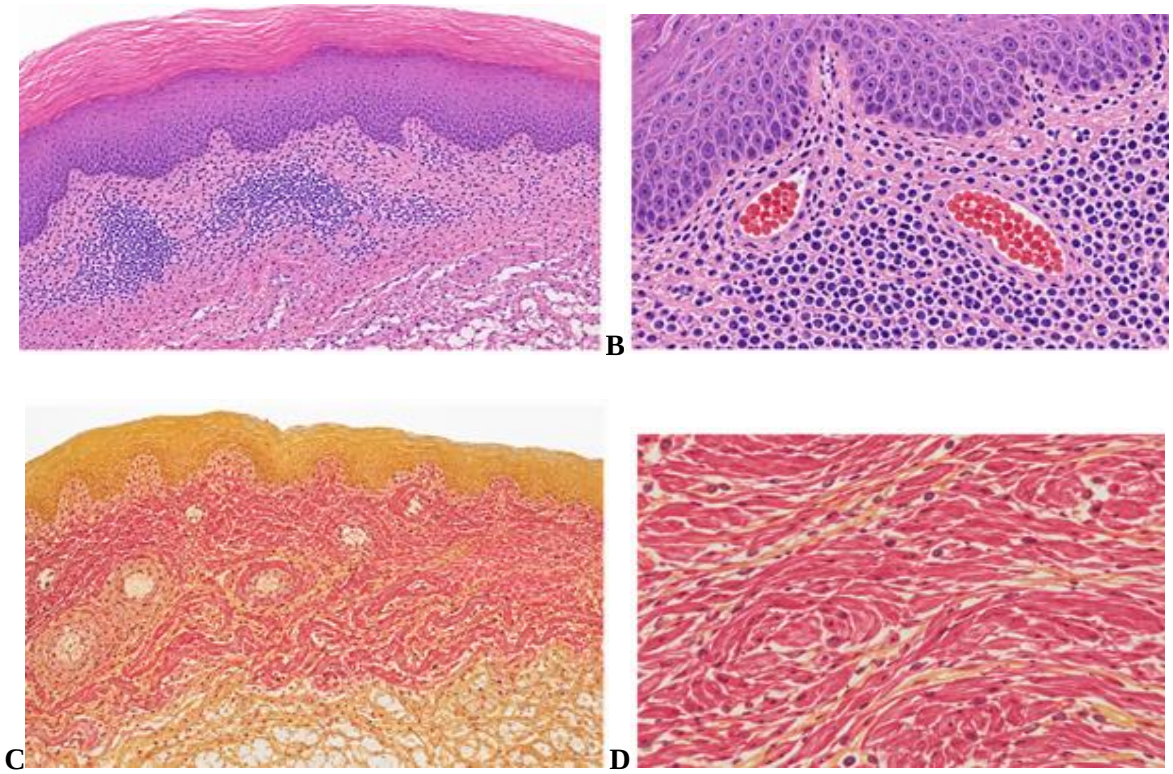
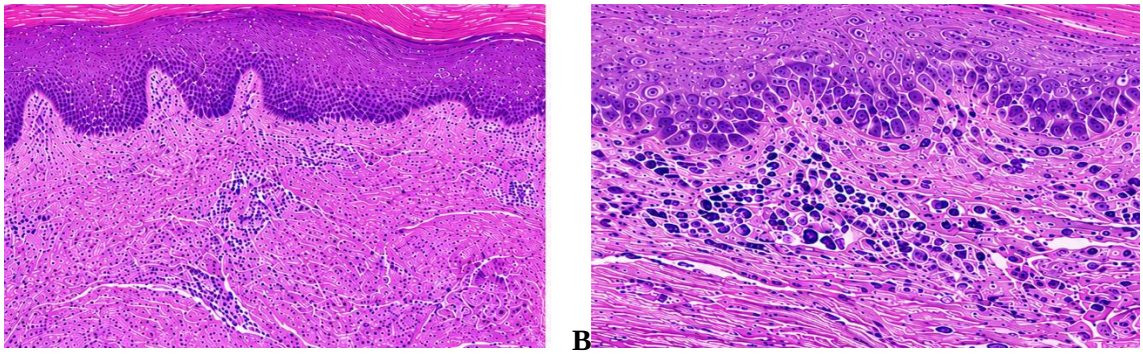


Figure 2. Histopathological alterations in conventional CS. **(A)** Hematoxylin-eosin stain (×100): diffuse epithelial hyperkeratosis and inflammatory infiltration. **(B)** Hematoxylin-eosin stain (×400): acanthosis, vascular congestion, and chronic inflammatory infiltrate. **(C)** Van Gieson's stain (×100): irregular collagen bundles. **(D)** Van Gieson's stain (×400): dense collagen deposition and early fibrosis.

In contrast, HTP users generally demonstrated preserved epithelial and stromal architecture (**Figure 3**). Mild focal hyperkeratosis was observed in 3 specimens (15.0%), epithelial acanthosis in 2 (10.0%), and epithelial atrophy in 1 (5.0%). Chronic inflammatory infiltration was present in 6 specimens (30.0%), while vascular congestion and stromal edema were identified in 4 (20.0%) and 3 (15.0%) specimens, respectively. Collagen fiber disorganization was present in 2 specimens (10.0%), whereas early fibrosis was identified in 1 specimen (5.0%).

No case of mild epithelial dysplasia was identified among HTP users, compared with 4 cases (20.0%) among CS participants. Although the observed absolute difference was 20.0 percentage points (95% CI, 2.5 to 37.5), the direct comparison did not reach statistical significance ($p = 0.106$), reflecting the small number of biopsy specimens and the limited number of dysplastic events. Accordingly, the absence of dysplasia in the HTP subgroup should not be interpreted as evidence that HTP exposure is associated with zero risk of epithelial dysplasia.

Representative findings in HTP users are presented in **Figure 3**. The majority of specimens showed preserved epithelial maturation and connective tissue organization, with only focal inflammatory and collagen alterations.



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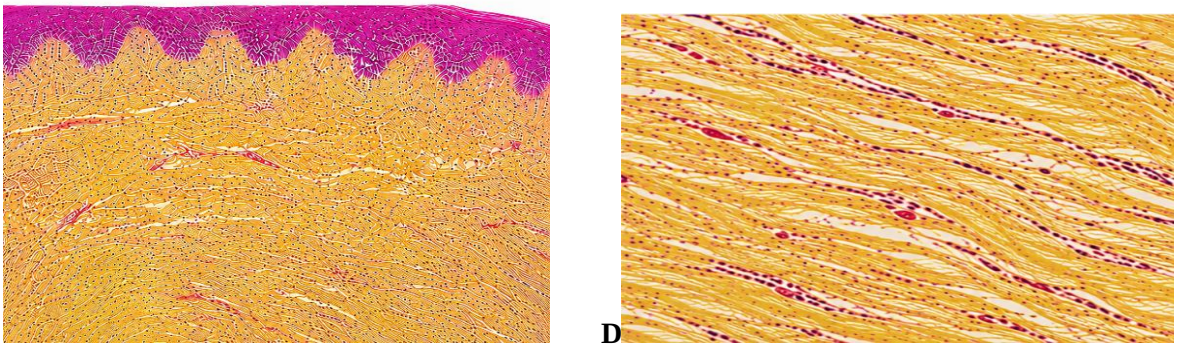

Figure 3. Histopathological alterations in heated tobacco product users.

(A) Hematoxylin and eosin staining, ×100, demonstrating largely preserved epithelial architecture with mild focal hyperkeratosis. **(B)** Hematoxylin and eosin staining, ×400, demonstrating mild inflammatory infiltration. **(C)** Van Gieson's picrofuchsin staining, ×100, demonstrating relatively preserved connective tissue organization. **(D)** Van Gieson's picrofuchsin staining, ×400, demonstrating minimal focal collagen remodeling without established fibrosis.

Overall, histopathological examination demonstrated a higher frequency of epithelial, inflammatory, vascular, and connective tissue alterations in the CS group than in the HTP group. The largest absolute between-group differences were observed for vascular congestion (+55.0 percentage points), collagen fiber disorganization (+55.0 percentage points), chronic inflammatory infiltration (+50.0 percentage points), hyperkeratosis (+45.0 percentage points), and epithelial acanthosis (+45.0 percentage points). These findings indicate substantially greater histopathological alteration among the biopsied CS participants. However, because the histopathological analysis was based on a small, randomly selected subset and the study was cross-sectional, the observed differences should be interpreted as associations rather than causal effects

4. DISCUSSION

The present study found that conventional cigarette smoking was associated with a significantly higher frequency of clinical and histopathological alterations of the oral mucosa than HTP use. Conventional cigarette smokers exhibited a higher prevalence of clinically detectable lesions, including diffuse erythema, hyperkeratosis, and leukoplakia, together with more pronounced epithelial hyperkeratosis, acanthosis, chronic inflammatory infiltration, vascular congestion, collagen disorganization, and early fibrosis. In contrast, HTP users exhibited only mild epithelial and stromal alterations, suggesting that although HTP use may be associated with less pronounced tissue alterations than conventional cigarette smoking, HTPs cannot be considered biologically harmless.

Our clinical findings are consistent with epidemiological studies demonstrating that conventional cigarette smoking remains one of the strongest risk factors for oral mucosal lesions, periodontal destruction, delayed wound healing, and oral potentially malignant disorders (OPMDs)12-13,16-17. Cigarette smoke contains thousands of chemical compounds, including polycyclic aromatic hydrocarbons, tobacco-specific nitrosamines, aldehydes, and reactive oxygen species, which can continuously irritate the oral epithelium and promote chronic inflammatory responses. The exclusive occurrence of leukoplakia and diffuse hyperkeratosis among conventional cigarette smokers in our cohort supports the concept that prolonged exposure to combustible tobacco products may induce adaptive epithelial responses that can precede the development of potentially malignant changes.

Histopathological examination demonstrated a higher frequency of epithelial and stromal alterations in oral mucosal biopsy specimens from conventional cigarette smokers. Hyperkeratosis, epithelial acanthosis, epithelial atrophy, and mild dysplastic changes were significantly more frequent among conventional cigarette smokers than among HTP users. Similar microscopic alterations have been reported in studies evaluating tobacco-associated oral lesions, in which chronic exposure to cigarette smoke was associated with abnormal epithelial differentiation, increased keratinization, and disruption of epithelial homeostasis17,18. These epithelial changes are considered early indicators of chronic mucosal injury and may reflect continuous adaptation to chemical and thermal irritation.

One of the most prominent findings of our study was the dense chronic inflammatory infiltration observed in the

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lamina propria of conventional cigarette smokers. Experimental studies have demonstrated that cigarette smoke can activate oxidative stress pathways, stimulate NF-κB signaling, and increase the expression of pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α16,19. Persistent inflammatory activation may contribute to extracellular matrix degradation, endothelial dysfunction, impaired tissue repair, and increased susceptibility to carcinogenic processes. The predominance of lymphoplasmacytic inflammatory infiltrates observed in our specimens is therefore consistent with the chronic inflammatory response described in molecular studies of tobacco-associated oral mucosal alterations.

An important observation of the present study was the marked connective tissue remodeling identified by Van Gieson's staining. Conventional cigarette smokers demonstrated collagen fragmentation, architectural disorganization, and early fibrosis significantly more frequently than HTP users. Cigarette smoke exposure has been shown to impair fibroblast proliferation, reduce collagen synthesis, increase matrix metalloproteinase activity, and alter extracellular matrix turnover through oxidative stress-related mechanisms21-22. These biological processes may explain the connective tissue abnormalities observed in our biopsy specimens and may indicate progressive stromal remodeling associated with chronic tobacco exposure. Although HTP users exhibited substantially milder histopathological alterations, microscopic evidence of tissue changes was nevertheless present. Mild focal hyperkeratosis, slight inflammatory infiltration, and limited collagen disorganization indicate that exposure to heated tobacco aerosols can be associated with biological responses in the oral mucosa. These findings are consistent with in vitro and clinical investigations demonstrating that aerosols generated by HTPs contain nicotine and other potentially harmful constituents, including formaldehyde, acetaldehyde, acrolein, volatile organic compounds, and ultrafine particles, which may contribute to oxidative stress, inflammatory cytokine production, and epithelial alterations despite generally lower concentrations of several toxicants than those generated by conventional cigarettes3,8,20,23. Consequently, lower exposure to selected toxicants should not be interpreted as an absence of biological risk. The comparatively milder histopathological alterations observed among HTP users in this study should be interpreted in the context of the study design. Importantly, HTP users had a history of previous conventional cigarette smoking. Therefore, the observed differences may reflect differences in current tobacco exposure as well as differences in cumulative exposure history and other participant characteristics. The present findings demonstrate an association between current tobacco-use category and the histopathological characteristics of the oral mucosa, but they do not establish that HTP use itself is responsible for the observed alterations or that switching from conventional cigarettes to HTPs definitively reduces long-term oral disease risk.

Heating tobacco at temperatures below those associated with combustion reduces the formation of many combustion-derived toxicants, including several carcinogenic compounds23,24. Nevertheless, HTP use continues to provide substantial nicotine exposure, and nicotine has been shown to influence keratinocyte proliferation, angiogenesis, inflammatory responses, and fibroblast metabolism20. Therefore, although switching from conventional cigarettes to HTPs may potentially reduce the magnitude of some tobacco-related tissue alterations, complete normalization of oral mucosal histology should not be expected. The strengths of this study include the combined clinical and histopathological evaluation of oral mucosal changes, together with assessment of connective tissue remodeling using both hematoxylin and eosin and Van Gieson's staining. Furthermore, the direct comparison of exclusive HTP users with conventional cigarette smokers provides additional evidence in an area in which comparative histopathological data remain relatively scarce. Several limitations should be acknowledged.

First, the cross-sectional design does not permit conclusions regarding causality or the progression of tissue alterations over time. Second, the relatively small number of biopsy specimens limits the statistical power to detect uncommon histopathological findings. This limitation was partly attributable to the high costs associated with histopathological processing and analysis.

Third, an important consideration is that all participants in the HTP group had previously smoked conventional cigarettes. Consequently, the HTP group cannot be considered a tobacco-naive population, and the observed oral mucosal findings may reflect both current HTP exposure and residual effects of previous combustible cigarette exposure. The present study therefore does not permit the effects of HTP use to be isolated from those associated with cumulative previous cigarette exposure. Longitudinal studies following individuals from the time of transition from conventional cigarettes to HTPs would be better suited to assessing changes in oral mucosal health following switching.

Finally, immunohistochemical and molecular biomarkers of proliferation, oxidative stress, inflammation, and extracellular matrix remodeling were not evaluated.

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Future longitudinal studies integrating histopathological assessment with molecular analyses, including Ki-67, p53, IL-6, TNF-α, matrix metalloproteinases, and oxidative stress biomarkers, would further clarify the mechanisms underlying tobacco-associated oral mucosal alterations.

CONCLUSION

In this cross-sectional study, conventional cigarette smokers demonstrated a higher frequency of clinically detectable oral mucosal abnormalities and more frequent histopathological alterations of the oral mucosa than exclusive HTP users. Conventional cigarette smokers exhibited a markedly higher prevalence of epithelial hyperkeratosis, acanthosis, chronic inflammatory infiltration, vascular congestion, collagen disorganization, and early fibrotic changes, whereas HTP users demonstrated comparatively milder epithelial, inflammatory, and connective tissue alterations. These findings indicate an association between current tobacco-use category and the characteristics of the oral mucosa, with greater clinical and histopathological alterations observed among conventional cigarette smokers. However, the findings should not be interpreted as evidence of causality or as definitive evidence regarding the long-term oral health effects of HTP use.

The presence of measurable epithelial, inflammatory, and connective tissue alterations among HTP users indicates that these products should not be considered biologically inert. At the same time, interpretation of the observed differences is limited by the cross-sectional design, the relatively small number of biopsied specimens, and the previous cigarette-smoking history of the HTP users. Therefore, the histopathological findings observed among HTP users cannot necessarily be attributed exclusively to current HTP exposure.

The observed relationship between clinical and histopathological findings supports the value of microscopic evaluation for characterizing tobacco-associated oral mucosal alterations that may not be clinically apparent. From a clinical perspective, regular oral examinations may be warranted for both conventional cigarette smokers and HTP users to facilitate the early identification of potentially malignant and other tobacco-associated oral mucosal lesions.

Further prospective longitudinal studies with larger, adequately powered cohorts are needed to clarify the long-term biological effects of HTP use and to determine whether oral mucosal alterations change following cessation of conventional cigarette smoking or transition to exclusive HTP use. Future studies should incorporate standardized assessment of tobacco exposure, appropriate histological control groups, blinded histopathological evaluation, adjustment for relevant confounding factors, and integrated molecular and immunohistochemical analyses. Ultimately, complete cessation of tobacco use remains the most effective strategy for reducing tobacco-associated oral health risks.

DECLARATIONS

Ethical Approval

Not applicable.

Competing Interests

The authors declare no conflict of interest.

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