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Original Article

Effect of different types of smoking on oral health and on cellular changes in oral mucosa

Amani Harrandah [a,*], Jumanah Bahatheq [b], Malaz Mandourah [b], Weaam Kaki [b], Alaa Alandonisi [b], Mayar Subahi [b], Rania Hanafi [a], Shahinaz Sembawa [c], Ibraheem Bamaga [a]

[a] Department of Basic and Clinical Oral Sciences, Umm Al-Qura University, College of Dentistry, Saudi Arabia
[b] College of Dentistry, Umm Al-Qura University, Saudi Arabia
[c] Department of Preventive Dentistry, Umm Al-Qura University, College of Dentistry, Saudi Arabia

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ABSTRACT

Objectives: Smoking is known to be a major risk factor for several diseases. Recently, electronic cigarettes have been introduced to the market; however, their effect on oral health has not been well studied yet. We aim to compare the effect of different types of smoking on oral health and to evaluate cytomorphological changes in oral mucosa among different types of smokers.

Study design: A total of 112 participants were recruited, conventional cigarette smokers (n = 28), electronic cigarettes smokers (n = 26), hookah smokers (n = 29) and non-smokers (n = 29). Oral examination, brush cytology and salivary flow test were performed. Cytological smears were stained and examined for cytomorphological changes. Oral symptoms, type, and frequency of smoking were obtained through questionnaires. SPSS program was used for statistical analysis.

Results: Most of the oral symptoms reported in this study were among conventional and electronic cigarettes smokers. While hookah smokers reported the least oral symptoms. Increase in DMFT and gingival index were observed among all smokers, mainly among conventional cigarettes smokers. Conventional cigarette and hookah smokers were found to have the most significant cellular changes. Electronic cigarette smokers had certain cellular changes as well.

Conclusion: All types of smoking adversely affect oral health and can induce cellular changes in the oral mucosa.

1. Introduction

Smoking has been associated with several oral conditions such as tooth stains, halitosis and impaired taste (Tubaishat et al., 2013; Khan and Mehmood, 2003). Furthermore, it has been associated with delayed wound healing, increased tooth mobility, bone loss, and xerostomia (Axelsson et al., 1998). Moreover, an increased risk of pre-cancerous lesions and cancers of mouth, pharynx, larynx and lip has been also linked to smoking (Winn, 2001).

Electronic cigarettes, initially introduced as cessation tools, have gained popularity, particularly among young adults due to their increased social acceptance, despite the American Dental Association's (ADA) recommendation against using them as a tobacco substitute (Manzoli et al., 2013; Chaffee, 2019).

Electronic cigarettes are devices with a heating element that generates aerosols. These aerosols might adhere to oral tissues causing hazardous effects. Furthermore, aerosol of liquids in electronic cigarettes share similar chemical and physical properties of gelatinous and high glucose food, which have been reported to be a risk of dental caries and induce changes in tissue properties (Frostell et al., 1967; Kim et al., 2018).

Surgical biopsy is the gold standard for diagnosing oral lesions; however, it is an invasive technique and may not be feasible in every clinical setting (Babshet et al., 2011). Brush cytology is a sensitive, fast, and noninvasive alternative technique (Seifi et al., 2014). Liquid-based cytology (LBC) is increasingly used in research for cytological assessments and cancer diagnostics but traditional smear methods remain prevalent in clinical settings (Seifi et al., 2014; Kaufman et al., 2020). In oral lesions, LBC has a significant specificity and sensitivity to diagnose premalignant and malignant lesions (Babshet et al., 2011).

Tobacco use has been reported to cause cytomorphological changes in oral mucosa such as, increase in nuclear-cytoplasmic ratio (Cowpe

* Corresponding author at: Department of Basic and Clinical Oral Sciences, Umm AlQura University College of Dentistry, Makkah, Saudi Arabia.
E-mail address: amharrandah@uqu.edu.sa (A. Harrandah).

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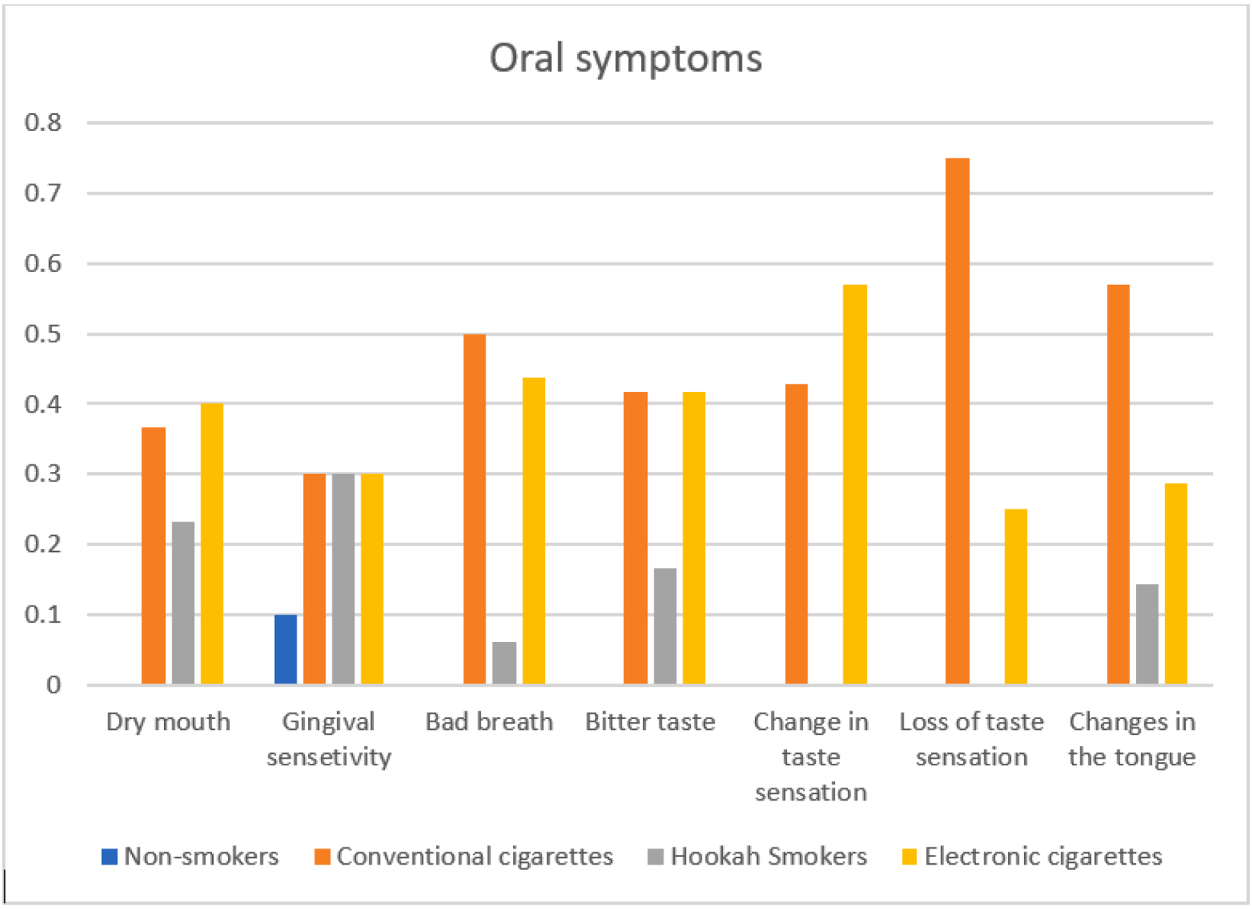

Fig. 1. Oral symptoms reported by different types of smokers. Comparison of different oral symptoms (dry mouth, gingival sensitivity, bad breath, change in the tongue, bitter taste, change in taste sensation and loss of taste sensation) in conventional cigarettes smokers, electronic smokers, hookah smokers and non-smokers.

Table 1
Clinical examination of Dental Caries using DMFT index.

DMFT INDEX	Smoking groups									
	Conventional cigarette		Electronic cigarette		hookah		Non-smokers		Total	
	N	%	N	%	N	%	N	%	N	%
Score 0	2	1.8	2	1.8	6	5.4	3	2.7	13	11.6
Score 1–4	2	1.8	4	3.6	1	0.9	4	3.6	11	9.8
Score 5–9	10	8.9	10	8.9	16	14.3	14	12.5	50	44.6
Score 10–14	8	7.1	9	8	4	3.6	7	6.3	28	25
Score 15–21	6	5.4	1	0.9	2	1.8	1	0.9	10	8.9

Table 2
Clinical examination of gingival condition using Gingival Index in relation smoking.

Gingival Index	Smoking Groups									
	Conventional Cigarette		Electronic Cigarette		Hookah		Non-Smokers		Total	
	N	%	N	%	N	%	N	%	N	%
Grade 0	5	4.5	8	7.1	6	5.4	24	21.4	43	38.4
Grade 1	5	4.5	7	6.3	17	15.2	4	3.6	33	29.5
Grade 2	13	11.6	9	8	6	5.4	1	0.9	29	25.9
Grade 3	2	1.8	2	1.8	0	0	0	0	4	3.6
Grade4	3	2.7	0	0	0	0	0	0	3	2.7
P Value	<0.001									

Table 3
Reduced salivary flow related to type of smoking.

Salivary flow rate	Smoking Groups									
	Conventional Cigarette		Electronic Cigarette		Hookah		Non-Smokers		Total	
	N	%	N	%	N	%	N	%	N	%
normal (1–3 ml/min)	21	18.8	14	12.5	19	17	29	25.9	83	74.1
low (0.9–0.7 ml/min)	1	0.9	3	2.7	2	1.8	0	0	6	5.4
Very low (<0.7 ml/min)	6	5.4	9	8	8	7.1	0	0	23	20.5
P Value	<0.001									

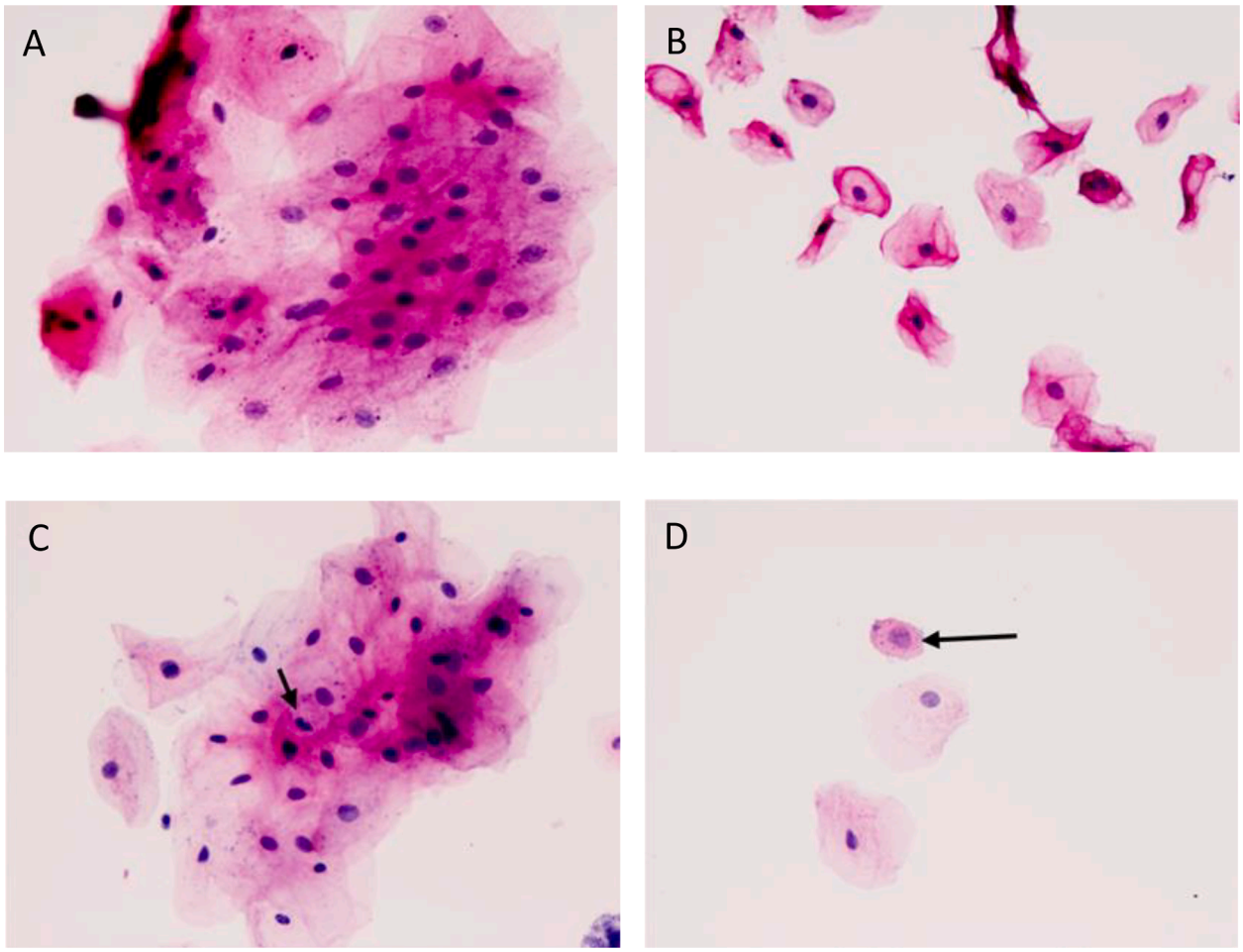

Fig. 2. Photomicrographs of oral cytological smear of buccal mucosa stained by (H&E). Cytological staining method showing different cellular changes. (A) A photomicrograph showing hyperchromatism (200X). (B) a photomicrograph showing cytoplasmic and nuclear pleomorphism (200X). (C) A photomicrograph showing hyperchromatism, nuclear pleomorphism, prominent nucleoli and mitosis(arrow) (Orig. mag. X200). (D) A photomicrograph showing increase nuclear cytoplasmic ratio (arrow) (Orig. mag. X200).

et al., 1988; Singh et al., 2014). These changes might be attributed to toxins and carcinogens found in cigarettes, intraoral pH changes, high temperature, mucosal dryness, and alteration of immune responses (Nazir et al., 2019).

Further studies are needed to investigate the effects of electronic cigarettes on oral health and its influence on cytomorphology of oral mucosa. Therefore, the aim of this study is to compare the effect of different types of smoking on oral health, including cytomorphological changes in oral mucosa.

2. Materials and methods

This is a cross sectional study, conducted between November 2019 and November 2020, and was approved by Institutional Review Board of Umm Al-Qura University (No.137-19).

2.1. Participants

A convenience sample of participants (aged 18 and 45 years old) who consented to participate in the study was collected from different cafes, shopping centers and educational institutions in Makkah province. Healthy participants who have been smoking for a duration longer than six months were included in the study. Pregnant women and participants with chronic diseases were excluded from this study. Informed consent was obtained from each subject.

To report different oral symptoms, an anonymous questionnaire was

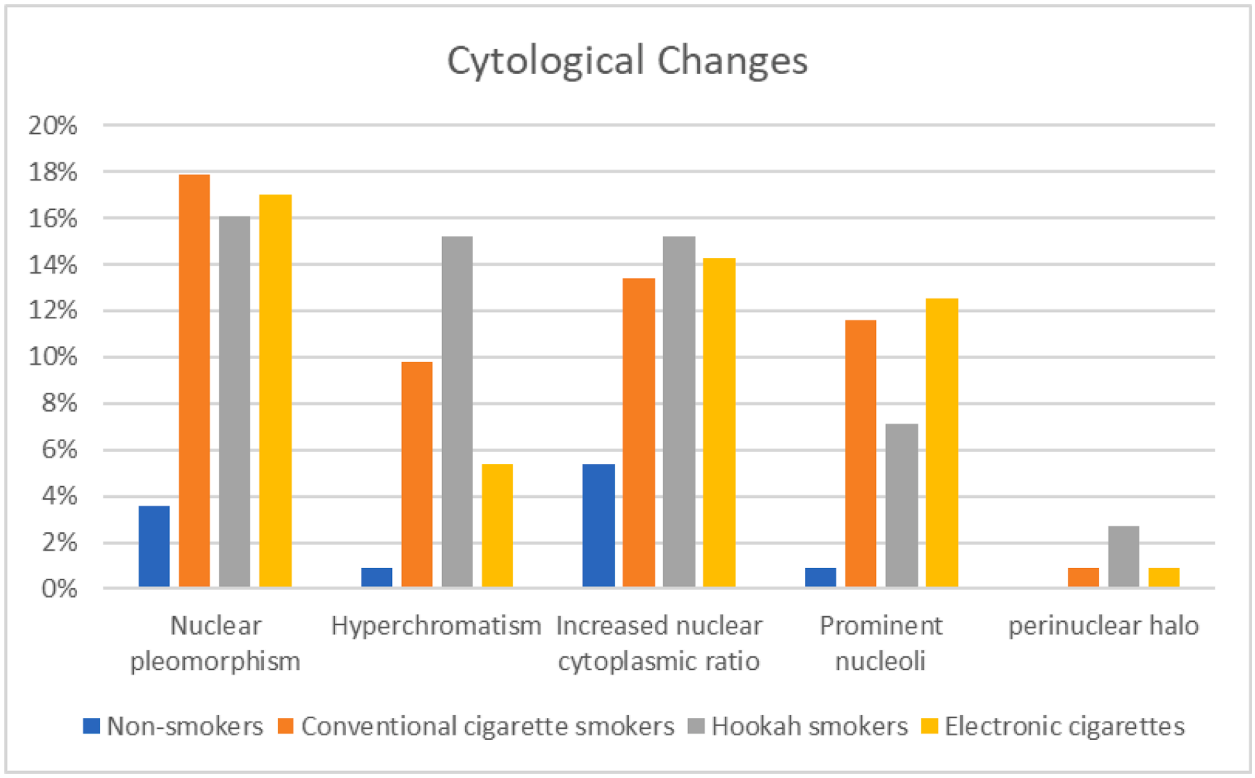

Fig. 3. Cytological changes observed in different types of smoking compared to non-smoking control. Comparison of different cellular changes including (nuclear pleomorphism, hyperchromatism, increase nuclear cytoplasmic ratio, prominent nucleoli and perinuclear halo) among conventional cigarettes smokers, electronic smokers, hookah smokers and non-smokers.

distributed to all participants. The questionnaire contained multiple choice and close-ended questions about their current health conditions, history of smoking and their oral complaints. Questionnaires were distributed to 15 subjects and was adapted according to their feedback.

2.2. Oral examination

A brief oral examination was performed to assess the presence of plaque or calculus, fluorosis, broken or stained teeth. In addition, oral mucosa was examined for any gross lesions. Dental examination was performed using Decayed-Missing-Filled index (DMFT) (Organization, 2013). While gingival health was assessed using gingival index (Löe, 1967). Disposable dental mirrors and explorers were used for examination on a dental chair. Four examiners were involved in the examination after being trained for using the above-mentioned indices for examination by their expert supervisor.

2.3. Salivary flow test

Salivary flow test was conducted using stimulated whole saliva - gum method (Navazesh and Kumar, 2008). Briefly, each participant was instructed to chew a piece of sugar-free gum for approximately 70 S per minute. After every minute, the subject was asked to spit saliva in a tube without swallowing and keep chewing. The first two-minute collection was discarded. By the third minute, the subject was asked to spit saliva into the tube.

2.4. Oral brush cytology

Cytological smears from buccal mucosa were taken using a sterile disposable cytological brush Cervex-Brush® - (Rovers Medical Devices, Oss the Netherlands). Using a constant medium pressure, the brush was stroked several times against buccal mucosa then immediately fixed in BD SurePath liquid-based pap test and preserved in 4 °C.

2.5. Slides preparation and staining

Samples were centrifuged at two different speed levels at 4 °C. The first cycle was for 2 min at 200 g. The second centrifuge cycle was for 10 min at 800 g. Next, cell pellet was re-suspended in 1000 µl PBS. Cells were transferred to a glass slide (Flex IHC microscope slide) using Cyto-Tek ® 2500 Cytocentrifuge adjusted at 15 × 100 rpm spun for five minutes. Samples were sent to King Abdullah Medical City (KAMC) histopathology laboratory for staining. Since screening for cellular changes and detection of atypia was aim of the study, H&E technique was used as convenient technique with high contrast and resolution for different cellular changes. Slides were stained within maximum of three days of preparation according to Hematoxylin and Eosin (H&E) cytological staining method.

2.6. Quantitative cytomorphometric evaluation

In each cytologic slide, cells were evaluated in three microscopic fields and examined at ×100 magnification. Smears were obtained from apparently normal mucosa without white, red colored lesions or ulcerated surfaces. Cytological smears were interpreted based on the following parameters: enlarged nuclei, variation in nuclear size and shape (pleomorphism), nuclear membrane irregularity, nucleocytoplasmic ratio and hyperchromatism. Percentage of cellular atypia will be obtained in relation to the total number of cells in each field. In case of absence of cellular atypia in cytological smears signs of inflammation were noticed and percentage were calculated.

2.7. Statistical analysis

Epitools online calculator (Sergeant, xxxx) was used to calculate

sample size based on 90 % power, 0.05 significance level, 95 % confidence and 5 % marginal error out of a large population. The sample size was estimated to be 300. Data was analyzed using SPSS (version 29, IBM Corp). Descriptive statistics, Chi-square test, Fisher's exact test and ANOVA test were used to analyze the data. Level of significance was set at ($p < 0.05$).

3. Results

A total of 112 participants consented to be included in the study, of which 59.8 % are male and 40.2 % are female with a mean age of 23 years (SD: 4.255). Distribution of smoking types was 25 % conventional cigarette smokers (n = 28), 23.2 % exclusive electronic cigarette smokers (n = 26), 25.9 % were hookah smokers (n = 29) and 25.9 % are non-smokers (n = 29). Frequency of smoking was also evaluated, 33.9 % of participants smoke more than 5 times per day (n = 38), 17 % of participants smoke 2–5 times per day (n = 19), 10.7 % smoke once a day (n = 12), 10.7 % smoke once a week (n = 12), and lastly 1.8 % (n = 2) smoke once a month.

A significant difference between male and female was found regarding type of smoking ($P < 0.001$). Interestingly, females comprised most non-smokers in this sample (72.4 %) and were more likely to smoke hookah (69 %). On the other hand, most conventional cigarettes smokers (89.3 %) and electronic cigarettes smokers (96.2 %) were males.

3.1. Oral symptoms

Multiple oral symptoms had been reported by smokers (Odum et al., 2012; AlSadhan, 2016). This study identifies various oral symptoms reported by smokers, including dry mouth, gingival pain, bad breath, bitter taste, taste sensation loss, and tongue changes (Fig. 1).

Dry mouth was reported by (26.8 %) of participants with a significant difference ($P < 0.001$) between the groups. Electronic cigarette smokers reported the highest percentage (10.7 %), followed by conventional smokers (9.8 %) and hookah smokers (6.3 %). Interestingly, dry mouth was not reported by non-smokers. Thus, might be a risk factor for dry mouth (Fig. 1).

Gingival sensitivity was reported by (8.9 %) of participants, equally among all types of smokers (2.7 %), and only (0.9 %) among non-smokers (Fig. 1).

Bad breath was reported by (14.3 %) of respondents, mainly from conventional cigarette smokers (7.1 %) and electronic cigarette smokers. (6.3 %), and only (0.9 %) of hookah smokers with Non-smokers did not report bad breath. A statistically significant difference was reported among the groups ($P < 0.001$). Thus, smoking, both conventional and electronic cigarettes, might be a cause for bad breath (Fig. 1).

When evaluated bitter taste was reported by (10.7 %) of participants with a significant difference ($P < 0.001$) between the groups, (4.5 %) of electronic and conventional smokers experiencing it, and 1.7 % of hookah smokers. No bitter taste was reported among non-smokers (Fig. 1).

Furthermore, alteration in taste sensation was reported by only (6.3 %) of participants, mainly by electronic cigarette smokers (3.6 %) and conventional cigarette smokers (2.7 %) ($P = 0.02$). Additionally, complete loss of taste sensation was reported by 3.6 % of participants, all of which were either conventional cigarette (75 %), or electronic cigarette (25 %) smokers (Fig. 1).

In this cohort (6.3 %) of smokers reported tongue changes, with (3.6 %) being conventional, (1.8 %) being electronic, and (0.9 %) being hookah smokers, while non-smokers did not report any changes (Fig. 1).

In conclusion, it seems that when comparing different types of smoking both conventional and electronic cigarettes smoking have the same effect on different oral symptoms.

3.2. Clinical examination

Several studies have shown that smoking has an adverse effect on both dentition and oral mucosa, including caries, staining, and oral lesion (Meraw et al., 1998).

Prevalence of dental caries among all four groups was examined using DMFT index (Table 1). Highest DMFT scores (15–21) were found among conventional cigarettes smokers (5.4 %). While DMFT scores ranging from (10–14) were scored mostly by electronic cigarettes smokers (8 %) followed by conventional cigarettes smokers (7.1 %). DMFT scores between (5–9) were mainly found in Hookah smokers (14.3 %). High DMFT scores among smokers suggest that smoking has an adverse effect on dental health.

Teeth staining was found in (21.4 %) of participants. The highest percentage was among conventional cigarettes smokers (9.8 %) followed by electronic cigarette smokers (7.1 %) and lastly (2.7 %) among hookah smokers. Only (1.7 %) of non-smokers has teeth staining and discoloration ($P < 0.001$). Examination of oral mucosa showed generalized dark mucosal pigmentations in conventional cigarette smokers (10.7 %), electronic cigarette smokers (5.4 %) and hookah smokers (1.8 %) with a statistically significant difference between groups ($p < 0.001$). It appears that smoking can cause teeth and oral mucosa discoloration.

Oral mucosa was examined for abnormal lesions, conventional cigarette smoking group was the only group to show abnormal lesions (1.8 %). White lesions were found on the ventral surface of the tongue bilaterally (0.9 %) and leukoedema on the buccal mucosa bilaterally (0.9 %).

Gingival Index was used to assess gingival condition in all groups (Table 2), with conventional and electronic cigarettes smokers having the highest scores. Non-smokers had the least scores. Grade 0 gingival index comprised (38.4 %) of participants, of which the majority were non-smokers (21.4 %). Grade 1 gingival index was observed among (29.5 %) of participants of which the majority were hookah smokers (15.2 %), while Grade 2 was observed among (25.9 %) with the majority being conventional cigarette smokers (11.6 %) then electronic cigarette smokers (8 %). Grade 3 was observed among (3.6 %) while Grade 4 was observed among (2.7 %) of which all were conventional cigarettes smokers. When analyzed, a statistically significant difference between groups ($P < 0.001$).

Salivary flow rate evaluation revealed that although most participants had a normal salivary flow rate (74.1 %), low salivary flow rate was detected among (20.5 %) of participants, of which (8 %) were electronic cigarettes smokers, followed by hookah smokers (7.1 %) and (5.4 %) were conventional cigarettes smokers, with a statistically significant difference between groups ($P < 0.001$) (Table 3).

In general, data from oral examination confirm that smoking has an adverse effect on oral health, including teeth, gingiva and salivary flow rate.

3.3. Cellular changes

It is well known that smoking can induce some cytological changes in oral mucosa (Seifi et al., 2014; Orellana-Bustos et al., 2004). In this study we reported cytological changes among different types of smoking compared to non-smokers.

Pathological evaluation of buccal mucosal smears revealed multiple cellular changes among smokers including nuclear polymorphism, hyperchromatism, increase in nuclear-cytoplasmic ratio, prominent nucleoli and perinuclear halo (Figs. 2 and 3).

Nuclear polymorphism was detected in (54.5 %) of participants, of which (17.9 %) were conventional cigarette smokers, (17 %) were electronic cigarette smokers and (16.1 %) were hookah smokers, and only (3.6 %) were non-smokers with a statistically significant difference between the groups ($P < 0.001$).

Hyperchromatism was observed in (31.3 %) of samples, with the majority in hookah smokers (15.2 %) and conventional cigarette groups

(9.8 %) and only (5.4 %) of electronic cigarette smokers ($P < 0.001$). All groups showed an increase in the nuclear-cytoplasmic ratio; the highest percentage among hookah smokers (15.2 %), followed by the electronic cigarette smokers (14.3 %), and lastly conventional cigarettes smokers (13.4 %) ($P = 0.007$).

Prominent nucleoli were observed in (32.1 %) of samples, with electronic cigarette smokers having the highest percentage (12.5 %), followed by conventional cigarettes smokers (11.6 %) and lastly hookah smokers (7.1 %), with statistically significant difference among the groups ($P < 0.001$). Perinuclear halo was observed in (4.5 %) of samples, of which the majority were hookah smokers (2.7 %).

Overall, it appears that all smoking can cause cytological alterations in oral mucosa.

4. Discussion

Several studies reported the harmful effect of smoking on dentition, oral mucosa, and salivary flow rate (Beklen et al., 2021; Ogden et al., 1990; Rad et al., 2010). Electronic cigarettes has gained popularity, particularly among young people (Manzoli et al., 2013). Unfortunately, the use of electronic cigarettes has become popular among Saudi citizens and all over the world (Tehrani et al., 2022; Khanagar et al., 2019).

Different forms of smoking may have varied effects on oral health. While some suggest that electronic cigarettes are less harmful than traditional ones, research on their impact remains limited. Therefore, understanding their effect on oral health effects is crucial.

In this study, we examined the effects of different smoking habits on oral health, including cytological changes and oral symptoms. Both electronic cigarette and conventional cigarette smoking were found to have similar adverse effects on oral health, with electronic cigarette users reporting higher instances of dry mouth and altered taste sensation, while conventional cigarette smokers reported more cases of bad breath, tongue changes, and taste sensation alterations.

Numerous additional studies have confirmed the same findings, demonstrating the negative impact of smoking traditional cigarettes on a variety of oral symptoms (Beklen et al., 2021; Ogden et al., 1990; Rad et al., 2010). Interestingly, in this study hookah smokers reported the least adverse effect on oral symptoms. This might be explained by the fact that hookah smoking is usually less frequent than other forms of smoking.

Moreover, smoking seems to affect the health of dentition and gingiva. A correlation between tobacco smoking and increased risk of dental caries has been reported (Jiang et al., 2019). Here, we observe an increase in DMFT among smokers, particularly those who consume conventional cigarettes, which is consistent with Gale et al findings. (Gale et al., 2017).

Smoking adversely affects gingival health (Jiang et al., 2019; Gale et al., 2017) leading to higher gingival index scores compared to non-smokers. Furthermore, smoking commonly known to stains teeth (Schwartz et al., 2003). In this cohort smokers had more stained teeth and mucous membranes than non-smokers, independent of type of smoking.

Conventional cigarettes and hookah smokers showed highest level of cytological changes including nuclear polymorphism, hyperchromatism, and prominent nuclei. Although electronic cigarette smokers had certain cellular changes, such as perinuclear halo, traditional cigarette smokers and hookah smokers were found to have the most significant cellular changes. These findings may stem from DNA damage induced by conventional cigarette smoking in oral keratinocytes (Schwartz et al., 2003).

All types of smoking negatively impact oral health, with conventional cigarettes and electronic cigarettes having the most detrimental effects. Further studies are needed to understand electronic cigarettes' effects.

5. Conclusion

It appears that all types of smoking have an adverse effect on oral health. Moreover, it is likely that different types of smoking can induce different oral symptoms.

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