

## Original Research Article

# Clinical and cytological findings in oral cavity of young shisha smokers and non-smokers — a comparative study

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**Abstract – Introduction:** Shisha, a form of smoking tobacco is known to be detrimental to oral health. Yet, the effects of shisha on oral health is not well documented. Hence, this study was undertaken to compare the clinical and cytological findings in oral cavity of young shisha smokers as compared to non-smokers. **Materials and methods:** A cross-sectional analytical study was undertaken among 60 subjects including 30 young shisha smokers and 30 non-smokers. Clinical examination was carried out to record gingival status, periodontal status, oral hygiene and dental caries experience. Exfoliative cytology was used to study the cytological changes of buccal mucosal cells. Data was analysed using SPSS version 22. Student's *t*-test, Fisher's exact test and odd's ratio was used for comparison.  $p < 0.05$  was considered statistically significant. **Results:** No significant differences were found between the groups with respect to proportion of subjects with gingivitis ( $p = 0.071$ ), erosion ( $p = 0.085$ ) and dental caries experience ( $p = 0.329$ ). However, shisha smokers had poorer oral hygiene and more severe gingival inflammation as compared to non-smokers with  $p = 0.026$  and  $p = 0.007$  respectively. The odds of having atypical cells in oral mucosa among shisha smokers was 3.76 times higher than the non-smokers. **Conclusion:** The findings of our study showed that young shisha smokers had more atypical cells in oral mucosa as compared to their non-smoking counterparts. However, longitudinal studies are needed to affirm the findings of this study.

## Introduction

Tobacco usage is a leading public health problem and a major cause of death globally [1]. Apart from its effects on systemic health, tobacco use can be detrimental to oral health. Cigarette smoking can increase the incidence and severity of oral cancer, periodontal diseases and peri-implantitis [2]. The causative role of tobacco use in the malignant disorders of oral cavity has been well established by various clinical and epidemiological studies [3]. Tobacco can be consumed either in the smoking or smokeless form [4]. Common smoking forms of tobacco include several types of cigarettes (manufactured, hand-rolled, filtered, unfiltered, and flavoured), cigars, and pipes [5]. and also the reasonably newer forms like water pipe smoking and vaping [6].

Water pipe or Shisha smoking has become a universal scourge, especially amongst adolescents and young adults including university, college and school students [7]. This could probably be due to the mistaken acceptance among many people that waterpipes are harmless with no dependence, and hence appraised as a good alternative for cigarettes. However, a

small number of studies have revealed high levels of toxic substances in water pipe smoke [8,9]. Aerosols materializing from waterpipes are an amalgamation of charcoal and tobacco smoke, thus exposing users to nicotine, carbon monoxide (CO), polyaromatic hydrocarbons, volatile aldehydes, and tobacco-specific nitrosamines [9–12]. It has been shown that during a lone 30–60 minute waterpipe smoking experience, users can inhale over 40 litres of smoke as compared to 1 litre or less for a solitary cigarette. Therefore, the amount of toxic materials generated during a single waterpipe smoking session is far greater than those in the smoke generated from a single cigarette [10].

Comparable to cigarette smoking, waterpipe smoking has also exhibited connection with numerous conditions within the oral cavity, including impaired inflammatory responses, susceptibility to candida infections, periodontal diseases, dry socket, premalignant lesions, and oral cavity cancer [7,13,14]. Though Shisha is a tobacco-related product, analysis on its possible deleterious effects on health has not been considered in depth like that of cigarette smoking. There is a strong need to document its ill effects on general and oral health, especially among young smokers, who are falling prey for the habit, in an increasing number, so that the use of

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waterpipe just as with other forms of tobacco can be regulated. Additionally, young shisha smokers need to be made aware of its consequences. Hence, this study was undertaken to assess the clinical and cytological changes within the oral cavity of shisha smokers, in comparison to non-smokers.

## Materials and methods

This was a cross-sectional analytical study. The study was approved by the institutional ethics committee (SEGiEC/StR/FOD/14/2017-18). A survey was conducted among our University students to identify exclusive shisha smokers, and they were invited to take part in the study. As the sample size did not reach the expected number, a few patients visiting our oral health centre, who satisfied the inclusion criteria, were also included. The non-smokers were also selected from among the students of our university, to match the age of shisha smokers. Sample size was calculated based on an expected difference of 30% between the groups, setting alpha at 0.05 and 80% power. About 54 subjects (27 in each group) were required in total. Hence we recruited 60 subjects, 30 shisha smokers, and 30 non-smokers, based on the inclusion criteria. For inclusion in this study, shisha smokers had to be male, individuals between 20 and 30 years of age, with the habit of using only shisha, at least twice per week for a minimum time of 30 minutes per session and at least from 2 years, without history of using any other form of tobacco or alcohol. Non-smokers were those who never had a history of smoking any form of tobacco or alcohol consumption. Subjects with a history of systemic diseases, exposure to passive smoking, and those who did not consent, were excluded. Informed consent was obtained from all the participants after the objective of the study was explained. The participants were given a proforma to fill in their demographic data and details pertaining to the duration, intensity and frequency of shisha smoking.

### Clinical examination

All the study participants were subjected to clinical examination. Intra oral hard tissue and soft tissue examination was carried out to record any abnormalities in soft tissues, number and condition of teeth present. Basic Periodontal Examination (BPE) was carried out using a CPI probe. Oral hygiene was recorded using Oral Hygiene Index- Simplified [15]. Gingival inflammation and bleeding was recorded using Gingival Index [16]. Dental caries experience was recorded using DMFT [17]. In addition to it, presence or absence of extrinsic stains and non- carious tooth surface loss was also recorded, using a standard proforma.

### Sample collection via exfoliative cytology

Exfoliative cytology was used for cytologic examination of the buccal mucosal cells. Participants were asked to rinse their mouth with distilled water, and right buccal mucosa was dried using sterile gauze. A cytobrush was used to collect the cells

from right buccal mucosa and the collected material was then smeared on a pre-coded slide. It was fixed immediately using Sprayfix<sup>R</sup> ( Ethanol; Leica, USA) sprayed at 6 inches distance from left to right covering the smeared area, with not more than two sprays. The code on the slide corresponded with the code on the proforma. The prepared smears were subjected to Papanicolaou stain using standard procedure [18] and analyzed microscopically for the evaluation of the cytomorphological features. They were classified according to Papanicolaou's classification (1960) as follows: Class I (normal): only normal cells observed; Class II (atypical): presence of minor atypia but no evidence of malignancy; Class III (intermediate): an in-between cytology (the cells display wide atypia that may be suggestive of malignancy but are not clear cut cancer and represent precancerous lesions or in situ carcinoma); Class IV (suggestive of cancer): a few epithelial cells with malignant characteristics or cells with borderline characteristics; Class V: positive cancer cells that are obviously malignant [19].

### Statistical analyses

The results were analyzed using SPSS version 22 (IBM Corporation, Armonk, NY, USA). The comparison of mean oral hygiene scores and mean DMFT between the groups was done using Student's *t*-test. The proportion of subjects with different stages of gingival inflammation, stains, non-carious tooth surface loss, atypical cells in mucosa were compared using Fisher's exact test. Odd's ration was calculated to find out the relative odds of having atypical cells between shisha smokers and non-smokers.  $p < 0.05$  was considered statistically significant.

## Results

### Demographic distribution

Our study examined 60 subjects, 30 shisha smokers and 30 non-smokers. The overall mean age of the subjects was  $23.03 \pm 1.5$  years with a minimum age of 20 years and maximum age of 29 years. No significant differences were present in the mean ages between shisha smokers and non-smokers with a *p* value of 0.746. All the participants of our study were males.

Regarding ethnic origins, about 13% were Malay, 30% were Chinese, 18% were Indians and 37% belonged to other races like Arabs, Yemenis *etc.* No differences were present in ethnicity among the two groups with  $p=0.712$ . Table I shows the demographic distribution of the study participants.

### Comparison of clinical soft tissue findings

No obvious clinical changes were seen in oral mucosa among shisha smokers and non-smokers. About 50% of the subjects in our study had gingivitis. Among them, about 60% were shisha smokers and 40% were non-smokers. No significant differences were found in the proportion of subjects with gingivitis between the two groups with  $p=0.071$ . However,

**Table I.** Demographic distribution of study participants.

Variable	Shisha smokers	Non-smokers	<i>p</i> value
Age	22.96 + 1.88 years	23.10 + 1.21 years	0.746
<b>Race</b>			
Malay	4 (13.3%)	4 (13.3%)	0.712
Chinese	7 (23.4%)	11 (30%)	
Indians	6 (20%)	5 (18.4%)	
Others	13 (43.3%)	10 (38.3%)	

**Table II.** Comparison of Gingival and Oral Hygiene scores between shisha smokers and non-smokers.

Variable	Shisha smokers	Non smokers	<i>p</i> value
<b>Gingivitis</b>			
Present	19 (63.3%)	12 (40%)	0.071
Absent	11 (36.7%)	18 (60%)	
<b>Severity of Gingivitis</b>			
Mild	8 (42.1%)	10 (83.3%)	0.007
Moderate	11 (57.9%)	2 (16.7%)	
Mean OHI-S score	2.23 + 1.33	1.45 + 1.30	0.026
<b>Extrinsic Stains</b>			
Present	9 (30%)	1 (3.3%)	0.006
Absent	20 (70%)	29 (96.7%)	

among the subjects with gingivitis, a significantly higher number of them had moderate inflammation in the shisha group as compared to the non-smokers with  $p=0.007$ .

There was a significant difference in the Oral Hygiene Index-Simplified scores. Shisha smokers had a mean score of  $2.23 \pm 1.33$  in comparison to non-smokers who had a mean score of  $1.45 \pm 0.62$  with  $p=0.026$ . Also, the proportion of subjects with stains were more in the shisha group with 30% as compared to just 3% in the non-smokers group with  $p=0.006$ . [Table II](#) shows the comparison of gingival and oral hygiene scores between shisha smokers and non-smokers.

### Comparison of clinical hard tissue findings

Overall, 19 subjects (30%) had caries in our study. Out of this, 11 were shisha smokers and 8 were non-smokers. This difference was not significant with  $p=0.405$ . Also, no difference was found in the mean caries experience between the two groups with  $p=0.329$  ([Tab. III](#)).

About 6 subjects in our study showed dental erosion. Among them, 5 were shisha smokers as compared to 1 non-smoker. This result was not significant with  $p=0.085$ . [Table III](#) shows the caries experience and erosion between shisha smokers and non-smokers.

### Comparison of cytological changes

In our study, we found only class I (normal) cells and class II (atypical) cells in the oral mucosa of our participants. A significant difference was seen in proportion of subjects having atypical cells between shisha smokers and non-smokers. Out of 30 shisha smokers, 11 (36.7%) exhibited atypical cells as compared to 4 (13.3%) non-smokers. An odds ratio of 3.76 was obtained, indicating that smokers had 3.76 times greater chances of having atypical cells in oral mucosal cells as compared to the non-smokers. [Table IV](#) shows the comparison of atypical cells among shisha smokers and non-smokers.

### Discussion

Water pipe, a popular type of smoking tobacco is known by several names like Shisha, Hookah, Hubble-Bubble, Narghile and Argile. Shisha smoking has been shown to have some oral effects like staining of teeth and restorations, reduced ability of smell and taste, periodontitis, peri-implantitis, pre-malignant lesions, oral cancer *etc.* [13,14]. However, most of these studies have been done on older adults with prolonged smoking habits. Hence, this study was undertaken among young shisha smokers, with relatively lesser duration of

**Table III.** Comparison of erosion and dental caries experience among shisha smokers and non-smokers

Variable	Shisha smokers	Non smokers	<i>p</i> value
<b>Caries</b>			
Present	11 (36.7%)	8 (26.7%)	0.405
Absent	19 (63.3%)	22 (73.3%)	
Mean DMFT	0.86+1.30	0.56+1.04	0.329
<b>Erosion</b>			
Present	5 (16.7%)	1 (3.3%)	0.085
Absent	25 (83.3%)	29 (96.7%)	

**Table IV.** Comparison of atypical cells among shisha smokers and non-smokers.

Variable	Normal cells	Atypical cells	Odd's ratio	Confidence interval		<i>p</i> value
				Lower	Upper	
Non-smoker	26 (86.7%)	4 (13.3%)	3.763	1.038	13.646	0.04
Shisha smoker	19 (63.3%)	11 (36.7%)				

exposure to shisha, to assess the clinical and cytological changes in the oral cavity. We included only male participants in our study to eliminate the confounding effect of gender on caries experience, gingivitis and cytologic changes in oral mucosa.

In our study, shisha smokers demonstrated poorer oral hygiene with more debris, calculus and extrinsic stains, as compared to the non-smokers. Some studies in the past by Prasad *et al.* [13], Khemiss *et al.* [21] and Natto *et al.* [22] have also shown higher dental plaque scores among shisha smokers as compared to non-smokers. Poor oral hygiene in shisha smokers can be attributed to certain substances like tar present in the shisha smoke, and also, their negligent attitude towards oral health and hygiene. A study found that shisha smokers visited dentists less frequently as compared to non-smokers [22].

Our study did not show any variability in the proportion of subjects with gingivitis among smokers and non-smokers. But, in terms of severity, more shisha smokers had moderate inflammation of gingiva as compared to non-smokers. This finding was in contrast to the findings from other studies which have found decreased gingival bleeding among shisha smokers, despite the presence of periodontitis [22–25]. Occurrence of periodontitis in shisha smokers could be due to impaired immune and inflammatory responses as seen in cigarette smoking. Also, local factors like plaque and calculus due to neglected oral hygiene may contribute to the severity of gingivitis and periodontitis. However, in our study, only two shisha smokers had shallow pockets (BPE code 3). This could be due to the fact that our study participants were young with a mean age of 23 years and had relatively lesser exposure to shisha smoking (mean of 2.7±0.6 years) as compared to participants in other studies.

In terms of hard tissue findings, no differences were found in caries experience between the two groups, in our study. A high caries experience with an increased severity among shisha smokers as compared to non-smokers was found in a study conducted by Amjed *et al.* [26]. It has been hypothesized in the past that severe periodontal disease among shisha smokers might increase the susceptibility for root caries. It is also reasonable to presume that the sugar added as a flavouring agent in shisha can contribute to caries development. However, there are no studies done to explore the association of sugar content in shisha with dental caries, and hence research is required in this direction. An interesting finding in our study was that more shisha smokers showed erosion of teeth as compared to non-smokers, though the difference was not statistically significant. Whether this erosion is caused by chemicals present in shisha smoke or due to some other mechanism, needs further exploration.

Though all our study participants exhibited clinically normal oral mucosa, exfoliative cytology showed notable changes in oral mucosal cells, with more number of atypical cells among shisha smokers as compared to non-smokers. These changes were seen despite the fact that the mean duration of exposure to shisha smoking among our study participants was 2.7 years. The changes were mainly in the form of hyperchromatic nuclei, multi-lobed nuclei and elongated nuclei. A study conducted previously by Seifi *et al.* [27] on oral mucosal cells among water pipe smokers showed an increased nuclear size, nucleus to cytoplasm ratio and a decreased cytoplasm size, as compared to non-smokers. It has also been found that waterpipe smoking was associated with a significantly higher frequency of micro nuclei and had greater genotoxicity as compared to cigarette smoking [28,29]. A study done on

cigarette smokers previously, with clinically normal mucosa, showed a greater nucleolar activity, suggesting that smoking influences the cellular activity of the mucosa [30].

Though some studies in the past have compared either the clinical or the cytologic effects of waterpipe smoking to cigarette smoking on oral mucosa, our study stands out as we have compared both clinical and cytological effects of exclusive waterpipe smoking with that of non-smoking in a vulnerable group, which is the young university students. However, the limitation of our study was that we did not use morphometric analysis to quantify these changes, as the objective of the study was not to evaluate the magnitude of changes, rather assess clinical and cytological changes if any, in the oral cavity of young shisha smokers as compared to non-smokers. So, we may have failed in reporting the actual magnitude of cytomorphometric changes. Also, the role of other factors like inflammation, infection, hot and spicy food *etc* in causing cytological atypia in apparently normal oral mucosa cannot be ruled out [31], and none of these factors were explored in the present study. Hence, more longitudinal studies are required to explore the effects of shisha smoking on cytological changes in oral mucosa.

## Conclusion

The findings of our study showed that young shisha smokers had more atypical cells in oral mucosa as compared to their non-smoking counterparts. However, longitudinal studies including more subset of risk factors, with a larger sample size, is needed to affirm the findings of this study.

## Authors contribution

P. Ramamurthy: Concept, design, Intellectual content, data analysis and manuscript writing. B. Fernandes: Designing of the study, data collection, manuscript writing. A. Tegginamani: Literature Review, Sample processing and interpretation. A. Rath: Literature Review, data collection, Manuscript editing. P. Sidhu: Literature review, sample collection, Manuscript writing. A. Termizi: Manuscript Review.

## Conflict of interest

The authors declare that there is no conflict of interest.

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## Ethical approval

Ethical approval was not required. obtained. SEGiEC/StR/FOD/14/2017-2018.

## Informed consent

This article does not contain any studies involving human subjects.

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