

To Cite:

Elmahdi FM, Alhejaili ME, Alahmadi AA, Altaher MJA, Alsaedi RN, Aeq MS, Ballaji MM, Altamimi HS, Alsebae RO, Aljohani AS, Alharbi AMM, Alahmadi AS, Alraddadi AF, Ballaji MB, Jamous YF. Cytological changes and ki-67 protein expression in oral mucosa among water pipe users in the KSA. Medical Science 2022; 26:ms478e2519.

doi: <https://doi.org/10.54905/disssi/v26i129/ms478e2519>

Authors' Affiliation:

¹Department of Histopathology, College of Medicine, Alrayan Colleges, Madinah, KSA

²Department of Medicine and Surgery, Faculty of Medicine, Alrayan Colleges, Madinah, KSA

³Department of Molecular Pharmacology, King Abdulaziz City for Science & Technology (KACST), Riyadh, KSA

***Corresponding author**

Department of Histopathology, College of Medicine, Alrayan Colleges, Madinah, KSA

Email: alfaris-sust@hotmail.com

Peer-Review History

Received: 12 October 2022

Reviewed & Revised: 17/October/2022 to 11/November/2022

Accepted: 15 November 2022

Published: 20 November 2022

Peer-review Method

External peer-review was done through double-blind method.

URL: <https://www.discoveryjournals.org/medicalscience>



This work is licensed under a Creative Commons Attribution 4.0 International License.

Cytological changes and ki-67 protein expression in oral mucosa among water pipe users in the KSA

Faris Merghani Elmahdi^{1*}, Majed Eid Alhejaili², Ahmed Ali Alahmadi², Majed Jamal Abu Altaher², Renad Naji Alsaedi², Mawaddah Sameer Aeq², Mayar Mohammed Ballaji², Huda Saeed Altamimi², Rawan Osama Alsebae², Ahmed Sulaiman Aljohani², Ahmad Meshal M Alharbi², Ahmed Saleem Alahmadi², Abdulrahman Fayeze Alraddadi², Maysan Bassam Ballaji², Yahya Fahad Jamous³

ABSTRACT

Objective: The aim of this study was to detect cytological changes and Ki-67 protein expression in oral mucosa among water pipe users. **Methodology:** A case control study was carried out in Saudi Arabia from January to October 2022, two cytological oral smear samples were taken from 500 volunteers 300 were water pipe users (case) and 200 were normal individuals (control) and were stained using the Papanicolaou staining procedure and immunocytochemical method to show the expression of Ki-67. **Result:** Inflammation, infection, atypia and keratinization were interpreted from Papanicolaou stains. The prevalence of cytological inflammation was higher among water pipe smokers (77/300, 25.6%) than among non water pipe users (12/200, 6%). Reverse cytological infection and atypical were higher in water pipe smokers than in control, 9% versus 4.5% and 4.3% versus 0.5%, respectively, while keratinization was only identified in water pipe users (3.6%) versus control groups. The Ki-67 protein is expressed at a higher level in water pipe users compared to non users. **Conclusion:** The study results concluded that water pipe users had more atypical cells and more KI-67 protein expression in the oral mucosa as compared to their non water pipe users' counter parts.

Keywords: Cytological changes, Ki-67 expression, Oral Mucosa, Water Pipe, KSA

1. INTRODUCTION

Tobacco use is directly responsible for approximately 85% of all cases of oral

and lung cancer. One major risk factor for oral cavity cancer is using tobacco products, including cigarettes and water pipes. According to the calculated toxicity, a single session of hookah smoking is equivalent to anywhere from one to fifty cigarettes (Albany et al., 2019). Smoking a hookah poses the same health risks as smoking cigarettes. In contrast, the possibility of contracting oral cancer as a result of smoking can be reduced if patients quit smoking at an early age. Oral cancer rates across the world have been shown to correspond with the prevalence of tobacco use (Ali et al., 2022).

Many Saudis, especially young people, have fallen ill from smoking in recent years (Ahmed et al., 2021). An alarming 40.8% of male secondary school students in northern Saudi Arabia are current smokers, according to a recent study, Cigarette's account for 67.3% of tobacco use, followed by shisha at 22.4%. About 39.8% of Saudi Arabian adolescents who were surveyed reported smoking cigarettes daily, with 29.6% saying they smoked fewer than five cigarettes per day (Ehizele et al., 2012).

Although shisha is a tobacco related product, its potentially harmful effects on health have not been analyzed to the same extent as those of cigarette smoking. There is a pressing need to record the negative effects of water pipe smoking on oral health, particularly among young people who are increasingly falling prey to the habit, so that it can be regulated like other forms of tobacco. Further, young people who smoke shisha need to be warned about the dangers of doing so. So, the goal of this inquiry was to look at the differences between the mouths of shisha smokers and people who don't smoke.

Several previous studies used Ki-67 immunohistochemical (IHC) markers as cell proliferative markers for oral cancer; this marker has been shown to be reliable as a proliferative marker because it is expressed during all active phases of the cell cycle (G1, S, G2 and mitosis) except the G0 phase (Scholzen & Gerdes, 2000). Even though water pipe tobacco smoking is linked with cigarette like unfavorable health consequences and its use is on the increase among young, it remains a vastly under explored study area in many aspects. Therefore, the purpose of this research was to evaluate the effect of smoking a water pipe on the cytomorphological and immunocytochemistry pattern of the oral epithelium.

2. MATERIALS AND METHODS

For this case control study, which was conducted from January to October 2022, 500 people in good health were chosen at random. Male residents of Saudi Arabia comprised the entirety of the study population. Participants in this study were all male. Cases included Shisha smokers (N = 300) and controls included non smokers (N = 200). Every person who took part in the study had two buccal smears taken from them (employing all necessary safety precautions and sample adequacy measures). With ages between the ages of 18 and 65 and a mean of 23 years, both patients and controls appeared to be in good health. Each person had their buccal smear and sputum samples taken. Both the cases and the controls were free of drinkers and smokers. A carefully organized questionnaire was used to gather personal information and other demographic details.

Sample collection

Buccal smear: A wooden tongue depressor was used to collect oral exfoliative cells from the buccal mucosa, which covers both cheeks and the dorsum of the tongue. The cells were then directly spread on two clean glass slides, immediately fixed in 95% ethyl alcohol while they were still wet. Buccal smears were sent to the histopathology lab at Rayyan College of Medicine in Saudi Arabia for staining and diagnosis.

Papanicolaou's Stain

Following fixation in ethanol, smears were hydrated for 2 minutes in a series of ethanol solutions, decreasing in strength from 95% to 70% to distilled water. Smears were stained with Harris's hematoxylin for 5 minutes to visualize the nuclei, rinsed in distilled water to remove any excess stain, differentiated in 0.5% aqueous hydrochloric acid for 10 seconds to remove any excess stain particles and finally rinsed in distilled water to halt the decolorization process. The smears were then dehydrated in ethanol concentrations ranging from 70% to twice 95% for two minutes at a time, after being dyed blue in alkaline water for four seconds. Following 2-minute incubation in Papanicolaou Orange G6 solution, a 95% ethanol rinse and 3-minute incubation in EA50 Papanicolaou staining solution, smears were examined for cytoplasmic staining. Finally, the smears were dehydrated in 95% absolute ethanol, cleaned in xylene and mounted on the DPX (distance polystyrene xylene).

Immunocytochemical Method

The smear was rinsed with PBS three times for three minutes each time. Each slice was treated with 0.3% hydrogen peroxide in methanol for 15 minutes to inhibit endogenous peroxidase activity and then rinsed three times with PBS. These antibodies (Abs) were utilized: Sections were incubated with a primary mouse monoclonal KI67 antibody (Gene Tech Company Limited, Shanghai,

China) at a working dilution of 1/100, at 37°C for 30 min; after two washes in PBS, sections were incubated with a secondary antibody, Chem Mate TM En Vision of +/HRP (Gene Tech Company Limited), at room temperature for 30 min, then washed three times in PBS. The immunoreactivity was detected using a 1/100 dilution of diaminobenzidine (DAB) (Gene Tech Company Limited, Shanghai, China) as the final chromogen for 10 minutes, followed by a 3-minute wash in DW. Finally, three minutes were spent counterstaining portions with hematoxylin, rinsed for 5 minutes in running tap water, dehydrated in alcoholic solutions, cleaned in xylene and mounted with DPX. Ki67 expression was only identified as a distinct brown cytoplasmic staining in epithelial cells and nuclei.

Cytological Assessment

All pap-stained smears from cases and controls were analyzed for cytopathological abnormalities. We looked for signs of infection, inflammation, keratinization and atypia. Cytological alterations were characterized by features including bi or multinucleation, uneven.

Ethical Consent

Prior to the specimen being obtained, each participant was requested to complete an ethical consent form in writing. The City of Science and Technology of King Abdulaziz (KACST) ethical committee created and approved the informed ethical consent form, Grant No AT-87.34.

3. RESULTS

The present study investigated 500 healthy individuals aged 18-64 years, with a mean age of 34 years. Most shisha smokers were in the age range of 31–40 years, followed by 41–50 years, representing 90/300 (30%) and 77/300 (25.7%). Most of the non smokers were in the age group of 18–30 years, followed by 31–40 years, constituting 90/200 (47%) and 45/200 (12%), in that order. As indicated in Table 1.

The analysis of the results of the Papanicolaou stain revealed the presence of four results with different percentages: Inflammation, infection, atypia and keratinization. Cytological inflammation was identified among 77/300 (25.6%) water pipe smokers, which is higher than non users (12/200, 6%). The reverse cytological infection and atypia were higher in water pipe smokers compared with non users, 9% to 4.5% and 4.3% to 0.5%, respectively and keratinization was detected only in water pipe users (3.6%) compared with control groups. The percentages of abnormal results were 42.7% in water pipe users and 11% in non users (p 0.041) (Table 2), Figure 5 and Figure 6.

About 8 of 13 (61.5%) of the atypical cytologic cases were seen in the age groups of 41–50 years. Most of the cases of inflammation and infection were seen in the age range 51–60 years, representing 31/77 (40.2%) and 10/27 (37%), respectively and most of the cases of keratinization were seen in the age group >60 years, representing 7/11 (63.6%), as indicated in Figure 2.

Most cases of cytologic atypia occurred in the duration ≤ 16-20 years, followed by ≥ 21 years and 11–15 years, representing 8/13 (61.5%), 3/13 (23%) and 2/13 (15%), respectively. The majority of inflammatory cases were associated with duration of 11–15 years, constituting 50/77 (64.9%). Most of the cases of infection were seen in the duration ≥ 21 years, representing 10/27 (37%). The majority of keratinization cases had durations of 6–10 years, 16–20 years and ≥ 21 years, constituting 3/11 (27%) for all, as shown in Table 3.

Water pipe use resulted in high Ki-67 expression (5%) and low Ki-67 expression in 285 (95% of the cases), whereas the control group had all low Ki-67 expression, as shown in Table 4, Figure 3 and Figure 4. Most cases of cytologic atypia and keratinization show high expression of Ki-67 (66.6%) and 33.3%, respectively. while infection and inflammation show low expression, as shown in Figure 2.

Table 1 Description of the study population by age

	Case (n=300)		Control (n=200)		P
Age (year)	N	%	N	%	
18-30	55	18.3	94	47	0.5
31-40	90	30	45	12	
41-50	77	25.7	32	16	
51-60	54	18	25	12.5	

>60	24	8	4	2	
-----	----	---	---	---	--

Table 2 Frequency of cytopathological changes among the study population.

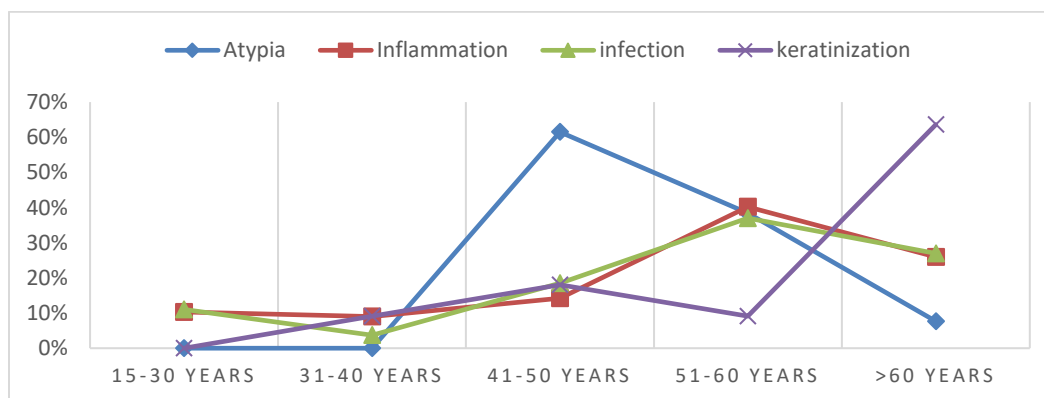
Papanicolaou staining result	Case (n=300)		control (n=200)		P
	N	%	N	%	
All diagnosed	128	42.7	22	11	0.041
Atypia	13	4.3	1	0.5	
Inflammation	77	25.6	12	6	
infection	27	9	9	4.5	
keratinization	11	3.6	0	0	

Table 3 Cytological changes according to duration of water pipe use by group

	≤ 5 years	6-10 years	11-15	16-20	≥ 21
Atypia	0(0%)	0(0%)	2(15.8%)	8 (61.5%)	3(23.1%)
Inflammation	10(12.9%)	5(6.5%)	50(64.9%)	5(6.5%)	7(9%)
Infection	4 (14.8%)	1(3.7%)	7(25.9%)	5(18.5%)	10(37%)
keratinization	0(0%)	3 (27%)	2(18.1%)	33 (27%)	33 (27%)

Table 4 Ki-67 expression among the study population

	Case (n=300)		control (n=200)		P
	N	%	N	%	
Ki67 Immunohistochemistry					
High Ki-67 (%)	15	5	0	0	0.045
Low Ki-67 (%)	285	95	200	100	


Figure 1 Description of the cytological changes in water pipe users by age.

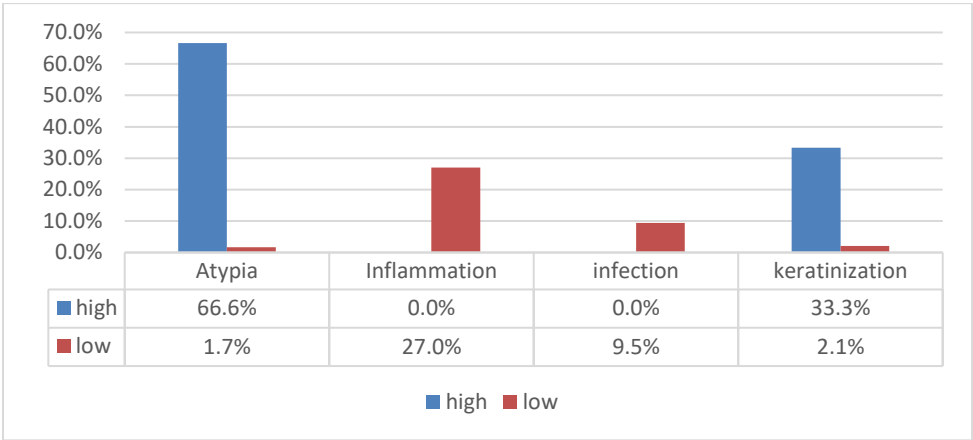


Figure 2 Relation of cytological changes in water pipe use to Ki-67 expression

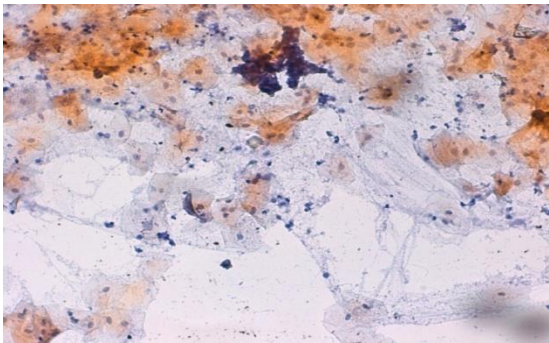


Figure 3 Photomicrograph of a buccal cell containing high Expression of Ki-67 (immunocytochemistry400x).

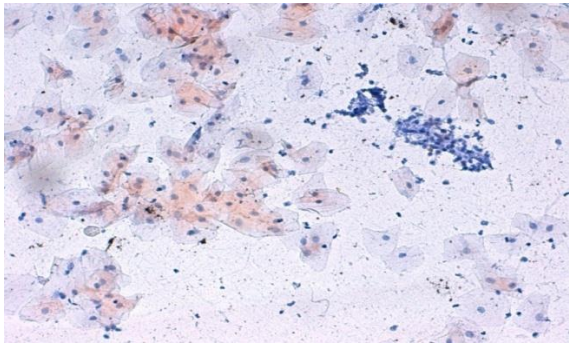


Figure 4 Photomicrograph of a buccal cell containing low expression of Ki-67 (immunocytochemistry400x)

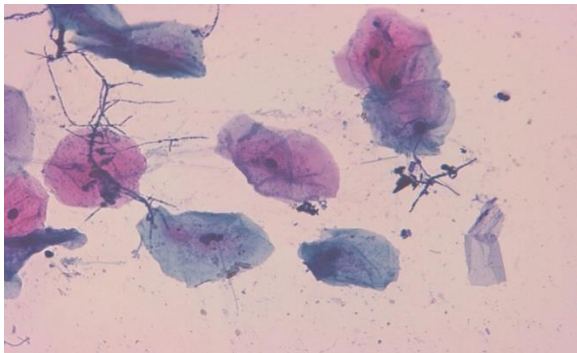


Figure 5 Photomicrograph of a buccal cell containing infection with moniliasis (Pap Stain 400x)

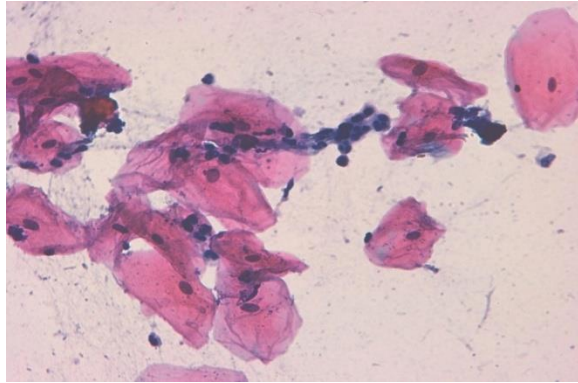


Figure 6 Photomicrograph of a buccal cell containing atypia (Pap Stain 400x)

4. DISCUSSION

Smoking shisha has been linked to a variety of oral side effects, including discoloration of teeth and restorations, reduced sense of smell and taste, periodontitis, periimplantitis, precancerous lesions, etc (Persson et al., 2001). Some studies have found that the risk of getting cancer is the same for smokers and water pipe users, while others have found that water pipe use is more dangerous than smoking (Shihadeh et al., 2004).

This study demonstrates the correlation between smoking shisha and oral epithelial alterations. These results revealed a high association between shisha use and the risk of oral epithelial atypical alterations, which can result in precancerous or cancerous changes in the mouth similar African research, such as the study by (Agabeldour et al., 2016) from Sudan, found greater rates. When they examined 70 people who regularly utilized water pipes, 47.1% exhibited cytomorphologically abnormal alterations, but none of the controls did. This little disparity between our results and theirs may be due to the fact that their sample size was less than ours (Agabeldour et al., 2016).

Studies have shown that smokers and people who use water pipes are more likely to have changes in their oral mucosal epithelia that could lead to cancer. In a cross-sectional study, cytologic smear samples were taken from the buccal mucosa, the tongue's lateral surface and the mouth's floor (right) of 40 smokers, 40 water pipe users and 40 normal people. They discovered that using a water pipe and smoking both cause some quantitative cytometric changes in the oral mucosa. However, smoking has a bigger effect on cytometric changes than using a water pipe (Seifi et al., 2014). Another study conducted in Jeddah, Saudi Arabia, indicated that cigarette smoking (with 65.6% prevalence) was the most common type of tobacco use, with water pipe use coming in at a distant second (38.1). Tobacco users had a higher prevalence of soft tissue lesions than the general population (88.8%) and nearly half of them had hairy tongues, smoker's melanosis, nicotine stomatitis, fissured tongues, gingival or periodontal inflammation and leukodema, as determined by traditional clinical examination. Erythroplakia (0.7%), smokeless keratosis (6.3%), oral submucous fibrosis (0.5%), leukoplakia (2.3%) and lichenoid lesions (0.4%) were the most common types of suspicious (premalignant) lesions, however, they affected only 0.5% of patients. The irritating effect of cigarettes on oral tissues may explain the high rate of oral mucosal soft tissue lesions (88%) (Al-Attas et al., 2014) however, the prevalence of cytological changes was higher in this investigation than in the prior report. The fact that there are cigar smokers and people who drink toombak, which has been linked to abnormalities in the oral epithelium, could be a reason.

Cases were more susceptible to infection and inflammatory conditions than controls. Nicotine and exposure of the respiratory tract epithelium to the irritating components of shisha (heavy metals such as arsenic, cobalt, chromium and lead) and carbon monoxide (CO) are the most important causative factors. A 35 to 50 percent increase in the incidence of respiratory infections and inflammation was associated with smoking. Recent studies support these conclusions indicating that tobacco use affects the proper growth and development of epithelial cells (Babu et al., 1996; Ganesh et al., 2018).

Furthermore, it was reported that the deteriorative effects increased with exposure duration. Similar findings have been reported previously for toombak dipping and cigarette smoking (Ahmed et al., 2003). And because tobacco is a peripheral vasoconstrictor, it affects the mouth wound's healing process. Carbon monoxide and other products of tobacco burning can restrict blood flow in the capillaries. According to a clinical trial, one cigarette can lower peripheral blood flow by 40% within one hour (Mayfield et al., 1998).

Ki67 protein expression is the most common marker of pathologic proliferation and early-stage tumor tissue changes in critical tissues. The present study showed shisha smokers have higher Ki67 protein expression, which is associated with cytological

changes. These findings are consistent with a study by Tabata et al., (2014), which reported that Ki67 expression was higher in smokers than non smokers.

This study also revealed that shisha use is more wide spread among persons under the age of 40, which may represent a future threat to a significant segment of the community. So, efforts to make smoking less of a problem and the use of screening programs are seen as important for controlling smoking in the future. Water pipe use is related to considerable oral epithelial alterations among Sudanese users, according to a study. People who smoke shisha should take part in regular screening programs to find conditions that could lead to cancer or are already cancerous. The exclusion criteria are one of the limitations of our research. In addition, the causes of the abnormal results, such as ki67 expression and its association with the kind, duration and age of smokeless tobacco use, cannot be determined by this research.

5. CONCLUSION

The oral mucosa can be altered effectively with the use of a water pipe. High Ki67 expression was seen in both atypia and keratinization. The presence or lack of Ki67 staining cannot be utilized to predict the prognosis of possibly malignant oral mucosal lesions, but these findings may indicate that Ki67 is involved in both the transformation of normal cells into cancerous ones as well as the proliferation of new cells.

Acknowledgement

We would like to thank Dr. Saad Ali Aljohani for his help and support, and we thank the participants who all contributed samples to the study.

Author Contributions

The authors really value the contribution of the study participants. Authors' contributions FE carried out the investigation and contributed to the manuscript's creation. The RA, MB, AMA, MEA and HA collections were in charge of sample collecting. MT, MBB, ASA, ASA, AAA, RA, MSA, AA and AFA carried out the laboratory work. YJ directed and participated in the study's planning, statistical analysis and manuscript writing processes. All writers reviewed and approved the final version.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Ethical approval

The study was approved by The City of Science and Technology of King Abdulaziz (KACST) Grant No AT-87.34.

Funding

This study has not received any external funding.

Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

REFERENCES AND NOTES

1. Agabeldour AA, Mohmed MI, Salih AA, Aljadoa AA, Ahmed HG. Assessment of Oral Epithelium Cytomorphological Changes among Water Pipe Smokers. Eur Academic Res 2016; (3):1172–11731.
2. Ahmed HG, Abboh EA, Alnajib AM, Elhussein GE, Binsaleh NK, Mustafa SA, Abdalla RA, Babiker AY, Mohammed MA. Is sputum cytology reliable for detection of atypical lung epithelial proliferative changes triggered by cigarette smoking? Int J Clin Exp Pathol 2021; 14(5):618-26.
3. Ahmed HG, Idris AM, Ibrahim SO. Study of oral epithelial atypia among Sudanese tobacco users by exfoliative cytology. Anticancer Res 2003; 23(2c):1943-9.
4. Al-Attas SA, Ibrahim SS, Amer HA, Darwish Zel S, Hassan MH. Prevalence of potentially malignant oral mucosal lesions among tobacco users in Jeddah, Saudi Arabia. Asian

- Pac J Cancer Prev 2014; 15(2):757-62. doi: 10.7314/apjcp.2014.15.2.757
5. Albangy FH, Mohamed AE, Hammad SM. Prevalence of smoking among male secondary school students in Arar City, Saudi Arabia. Pan Afr Med J 2019; 32:156. doi: 10.11604/pamj.2019.32.156.18558
6. Ali D, Al-Ahmari F, Mikami T, Baskaradoss JK. Increased expression of advanced glycation end products in the gingival crevicular fluid compromises periodontal status in cigarette smokers and water pipe users. BMC Oral Health 2022; 22(1):206. doi: 10.1186/s12903-022-02240-z
7. Babu S, Bhat RV, Kumar PU, Sesikaran B, Rao KV, Aruna P, Reddy PR. A comparative clinic pathological study of oral sub mucous fibrosis in habitual chewers of panmasala and betelquid. J Toxicol Clin Toxicol 1996; 34(3):317-22. doi: 10.3109/15563659609013796
8. Ehizele AO, Azodo CC, Ojehanon PI, Akhionbare O, Umoh AO, Adeghe HA. Prevalence of tobacco use among dental patients and their knowledge of its health effects. Niger J Clin Pract 2012; 15(3):270-5. doi: 10.4103/1119-3077.100619
9. Ganesh D, Sreenivasan P, Öhman J, Wallström M, Braz Silva PH, Giglio D, Kjeller G, Hasséus B. Potentially Malignant Oral Disorders and Cancer Transformation. Anticancer Res 2018; 38(6):3223-9. doi: 10.21873/anticancer.12587
10. Mayfield L, Söderholm G, Hallström H, Kullendorff B, Edwardsson S, Bratthall G, Brägger U, Attström R. Guided tissue regeneration for the treatment of intraosseous defects using a biabsorbable membrane. A controlled clinical study. J Clin Periodontol 1998; 25(7):585-95. doi: 10.1111/j.1600-051x.1998.tb02492.x
11. Persson L, Bergström J, Ito H, Gustafsson A. Tobacco smoking and neutrophil activity in patients with periodontal disease. J Periodontol 2001; 72(1):90-5. doi: 10.1902/jop.2001.72.1.90
12. Scholzen T, Gerdes J. The Ki-67 protein: From the known and the unknown. J Cell Physiol 2000; 182(3):311-22. doi: 10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9
13. Seifi S, Feizi F, Mehdizadeh M, Khafri S, Ahmadi B. Evaluation of cytological alterations of oral mucosa in smokers and water pipe users. Cell J 2014; 15(4):302-9.
14. Shihadeh A, Azar S, Antonios C, Haddad A. Towards a topographical model of narghile water pipe café smoking: A pilot study in a high socio-economic status neighborhood of Beirut, Lebanon. Pharmacol Biochem Behav 2004; 79(1):75-2. doi: 10.1016/j.pbb.2004.06.005
15. Tabata K, Tanaka T, Hayashi T, Hori T, Nunomura S, Yonezawa S, Fukuoka J. Ki-67 is a strong prognostic marker of non small cell lung cancer when tissue heterogeneity is considered. BMC Clin Pathol 2014; 14:23. doi: 10.1186/1472-6890-14-23