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The Effect of Cigarette and Hookah Smoke on Oral Bacterial Growth, *Streptococcus mutans* and *Streptococcus sanguis*: An In vitro Study

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Abstract

Background: *Streptococcus mutans* and *Streptococcus sanguis* are two important bacteria of the oral microflora. *S. mut*ans is the most important cause of dental caries and *S. sanguis* plays an important role in formation of microbial plaque.
Objectives: The present study aimed to determine the effect of cigarettes and hookah on thebacterial growth of *S. mutans* and *S. sanguis*.
Methods: The standard strains of *S. mutans* (PTCC = 1683) and *S. sanguis* (PTCC = 1449) were cultured on blood agar and incubated for 48 hours in different environments, including atmospheric air, carbon dioxide, three types of cigarette smoke (Winston, ultralight Winston, and Kent), and fruity tobacco. Then, the diameter of colonies was measured and data was compared using statistical tests, such as one-way ANOVA.
Results: Bacterial growth was 80%, 100%, 61%, 48%, and 59% in carbon dioxide, Winston, Kent, Ultralight Winston, fruit tobacco, respectively, for *S.mutans* ($P < 0.001$) and 134%, 38%, 169%, 105%, and 61%, respectively, for *S. sanguis*, which were higher than ordinary air ($P < 0.001$), in addition, the growth of *S. mutans* was more than *S. sanguis* in the Winston group.
Conclusions: These findings showed that the growth of *S. sanguis* was significantly greater than *S. mutans* in all groups, except in the Winston group.

Keywords: Cigarette Smoking, Tobacco, *Streptococcus mutans*, *Streptococcus sanguis*

1. Background

The prevalence of smoking and tobacco use is increasing in the world (1, 2). In Iran, about 12.5% of the adult population are daily cigarette smokers with a mean age onset of 20.5 years, while the prevalence of hookah smoking is 2.7% with an average of 3.5 times a day (3).

Tobacco smoking, the most of which is through cigarettes, increases the risk of all-cause mortality, and is responsible for 90% of causes of cancer, cardiovascular diseases, ischemic heart disease, chronic obstructive pulmonary disease, and stroke (4, 5). In addition it is the first cause of preventable death with more than 4000 compounds and 40 carcinogens, mainly nicotine and carbon monoxide (6). Despite the known hazards of smoke and various harm-reduction strategies and policies to reduce the rate of smokers (7), the prevalence of smoking is increasing around the world, estimated at 1.5 billion in 2015 (8), which is postulated to be due to underestimating the personal harm of smoke (9). Despite some advertisement on less harmful effects of hookah, it is proven that it is not only similar to cigarettes regarding nicotine exposure, it has greater CO, and smoke exposure (10).

In addition to the increased risk of bacterial and viral infections, such as pneumonia and peptic ulcer, it also increases the risk of periodontal diseases (11) and subgingival calculus deposition (12); second-hand smoke is also suggested to increase the risk of dental caries in children (13), which is hypothesized to be due to the immunological response and deteriorating effects on microbial flora (14).

Studying the oral microbial flora introduced streptococci as the main group of early colonizers in the oral biofilm with a fundamental role in the development of oral diseases (15). Studying the subgingival microbiology assessment has identified *Streptococcus (S.) mutans* as the main cause of dental decay; *S. sanguis* also plays a role in the oral plaque (16). The risk of respiratory infections also

increases in smokers, possibly due to provision of an environment for bacterial colonization (17), especially in sterile sites, such as trachea and altered epithelial secretion and inflammation (18).

Considering the increased risk of periodontal diseases, and the major role of *S. mutans* and *sanguis* in oral diseases, studies have investigated the effect of smoking on the growth of these two bacteria (19).

2. Objectives

Considering the increasing rate of cigarette and hookah abuse in Iran and the small number of studies on the bacterial agents responsible for tooth decay, the present study aimed to determine the effect of cigarette and hookah on the bacterial growth of *S. mutans* and *S. sanguis* in order to provide additional clinical proofs for cutting cigarettes and hookah in case of increased periodontal diseases by overgrowth of *S. mutans* and *sanguis*.

3. Methods

This present experimental laboratory study (in-vitro) is done in the immunology of infectious diseases research center, Rafsanjan University of Medical sciences, Rafsanjan, Iran from January to May 2018. *S. mutans* (PTCC = 1683) and *S. sanguis* (PTCC = 1449) that were prepared from the industrial and infectious bacteria and fungus of Iran were used (PTCC). Initially, these microorganisms were kept at 37°C for 24 hours in liquid medium of Tryptic Soy Broth (Merck KGaA, Darmstadt, Germany) for growth. Then, they were linear cultured on solid blood agar (Merck KGaA) and kept at 37°C for 72 hours, so colonies be identified and separated; next, a single colony of this miro-organism was taken and transferred to sterile physiologic serum (prepared at laboratory).

Bacteria were prepared at 0.5 U McFarland and the swab was dipped with the bacteria and cultured on disposable plates (Padtan Teb, Tehran, Iran) containing blood agar medium by spread plate method so that bacterial colonies grow separately on the medium; then, cultured plates were kept still for 2 - 5 minutes to demoisturize and let the bacteria stay on the surface. Plates were coded and transferred to the respective containers. A minimum of 50 colonies per strain was estimated as sample size using Cohen's table. For each strain of bacteria, 30 blood agar plates (60-well plates for both strains) were used. Six containers included three types of cigarettes, including conventional Winston® (Winston-Salem, North Carolina, US) containing 9 mg and 12 mg tar, ultra-light Winston® (Winston-Salem, North Carolina, US) containing 4 mg and 4 mg tar, and Kent® (Kent, Istanbul, Turkey) containing 0.7 mg and 8 mg tar, fruity hookah (Thunder Company, Shiraz, Iran), normal air, and carbon dioxide (CO_2). A total number of 25 mediums, 5 in each group, were selected. For preparation of cigarettes' smoke, a compartment with doors and two inner and outer taps were connected to the inlet valve via a converter; the outer tap was evacuated through connector converter to a vacuum pump and the smoke of half of the cigarette's length was entered to the container, devoid of normal air. Hookah smoke was prepared in the same way from fruity hookah for 1 minute; conventional air was selected as negative control and CO_2 as positive control. Then, the containers were kept at 37°C for 48 hours and the diameter of the grown bacteria was measured under loop microscope by caulis (Asim Instruments, Sialkot, Pakistan) with an accuracy of 0.01 mm and recorded and compared between the groups as the main outcome of the study. In the end, the data collected in the groups studied were compared statistically.

3.1. Statistical Analysis

The collected data was analyzed using SPSS software version 21. Values for diameter of the grown colonies (mm) were reported by minimum, maximum, and mean ± standard deviation (SD). Kolmogorov-Smirnov test was used to assess the normal distribution of data, which showed normal distribution of colony size distribution (mm) in different mediums and Levene's test was used to measure the variance difference, which showed homogeneous variance in the size of the colonies (mm) in different mediums ($P > 0.05$). Thus, bacterial growth was compared among the groups by One-way ANOVA; in case of significant difference, Tukey's multiple comparison test was used to determine the effect of different mediums. The mean diameter of the grown colonies (mm) were compared between two types of bacteria using independent two-sample t-test. The significance level of all statistical tests were considered at 0.05.

4. Results

In this study, the growth rate of *S. mutans* and *S. sanguis* increased in all mediums, compared to air, which, in turn, increased the size of colonies (mm) at different values in the mediums. Bacterial growth was 80%, 100%, 61%, 48%, and 59% in carbon dioxide, Winston, Kent, Ultralight Winston, fruit tobacco, respectively, for *S. mutans* ($P < 0.001$ (and 134%, 38 %, 169%, 105%, 61%, respectively, for *S. sanguis*, which were higher than ordinary air ($P < 0.001$). Mean, SD, minimum, and maximum values of mean colony diameter is compared among the 5 groups in Table 1. As

demonstrated, there was a significant difference between the colony diameters of both bacteria among the groups.

Further analysis showed that mean size of colonies (mm) of *S. sanguis* was significantly different between all groups pairwise ($P < 0.05$) (Table 1), while mean size of colonies (mm) of *S. mutans* was not significantly different between the other two groups ($P > 0.05$) (Table 1).

Comparison of the mean diameter of the grown colonies (mm) in different mediums between the two types of bacteria in Table 2 showed significantly more bacterial growth of *S. mutans* in all mediums ($P < 0.001$), except in Winston cigarettes ($P > 0.108$).

5. Discussion

The present study compared the bacterial growth of *S. mutans* and *S. sanguis* in 5 groups, including the smoke of Kent, Winston, ultralight Winston, fruity hookah, CO_2, and air, each on 5 cultural plates and the results indicated significantly greater bacterial growth of both bacteria in all mediums than air. In addition, the growth of *S. sanguis* was significantly greater than *S. mutans* in all mediums, except Winston. Despite the fact that nicotine concentration of Kent is less than the two others, the growth diameter of both microbes was greater in Kent, which indicates the role of some other factors rather than nicotine.

Similar in-vitro studies have evaluated the effect of the smoke of cigarette on bacterial growth of *S. mutans* and *S. sanguis*, which have resulted in diverse results. Research on the pure effect of nicotine on *S. mutans* and *sanguinis* showed significantly increased growth of *S. mutans*, however, not *S. sanguinis* in dual-species biofilm (20). Other researchers have also demonstrated the effects of nicotine on bacterial overgrowth of oral pathogens, including Streptococcus species (21), which is in line with the present study on the effect of nicotine on the overgrowth of *S. mutans*, although the present study investigated the effect of the smoke of cigarettes for better synchronization with the clinical settings of a human smoking a cigarette.

The brands investigated in the present study is similar to previous Iranian studies, which confirms that these brands are the most commonly used brands in Iran. Zonuz and colleagues studied the growth of *S. mutans* (ATCC 25175) and *S. sanguis* (ATCC 10556) on atmospheric air, CO_2, and cigarette smoke (Kent gold, Kent lights, and Bahman) and the results of this study indicated significantly increased the growth of *S. mutans* in cigarette smoke and CO_2 with the greatest impact of Bahman. They have also reported *mutans*/*sanguis* ratio of 1, 0.84, and 0.98 in Kent gold, Kent lights, and Bahman, respectively (19). The results of this study is similar to the present study, regarding greater growth of streptococcus in cigarette smoke, and CO_2, however, the growth of *S. mutans* was greater in their study than *S. sanguis*, while in the present study, it was vice versa. This difference could be due to the fact that we have selected a different brand of cigarettes and hookah, which have different concentrations of nicotine and carbon monoxide. In another Iranian study, Ebrahimi and coworkers compared the effects of atmospheric air, micro-aerophillic, CO_2, cigars, and three type of cigarette smoke (Winston, ultralight Winston, and Kent) on the growth of *S. mutans* (ATCC25175) and *S. sanguis* (ATCC10550) and reported that the growth of both bacteria by cigar and cigarette smoke significantly increased, with greater growth of *S. mutans* in all groups (22). The smoke of cigarette investigated in their study was similar to the brand studied in the present study and their results are consistent with the results of the present study on the increased growth of *S. mutans* and *S. sanguis* in all groups. However, the greater growth of *S. mutans* than *S.sanguis* is in contrary to the results of the present study, which could be affected by the methods of preparation of pure smoke of the cigarettes; as in the current study, we reconstructed the way humans smoke a cigarette to identify the results of smoking on bacterial overgrowth. Although, the results of the present study was not in line with previous studies regarding the type of the bacteria grown more, which is clinically not important, the clinical importance lies under this fact that all the above-mentioned studies, parallel to the results of the present study, have shown greater bacterial overgrowth of *S. mutans* than *S. sanguis* in cigarettes, and CO_2. According to the evidence, cessation of smoking can significantly reduce this bacterial overgrowth (23). Therefore, it is suggested that further strategies be implemented to reduce the rate of smokers (cigarette and hookah) to decrease the rate of oral diseases.

Regarding the effect of hookah on bacterial overgrowth of *S. mutans* and *S. sanguis*, as far as the authors are concerned, no in-vitro study has addressed this issue and this study is the first study in this regard, although studies have studied the association of hookah smoking on oral and periodontal diseases (24-26). Although hookah is smoked in some countries, its use is very prevalent among Iranians, especially youths (27). Thus, while the nicotine content and plasma levels are not different between cigarette and hookah (10), the concept of the general population is that hookah has less harms, and it is therefore used frequently in Iran, especially in women (28), which according to the results of the present study can have deteriorating effects on the oral health of women and their children, who are in close contact with their mother. Therefore it is necessary to emphasize the effect of hookah smoking on bacterial overgrowth of the oral cavity in future studies and include educational programs for Irani-

Table 1. Comparison of Mean Diameter of the Grown Colonies (mm) in Different Mediums

Variables	Kent[a]	Winston[a]	Ultralight Winston[a]	Hookah[a]	Air[a]	CO_2[a]	P Value
Streptococcus mutans	1.52 ± 0.08 (1.4 - 1.6)	1.88 ± 0.08 (1.8 - 2.0)	1.40 ± 0.07 (1.3 - 1.5)	1.5 ± 0.07 (1.4 - 1.6)	0.94 ± 0.11 (0.8 - 1.1)	1.7 ± 0.07 (1.6 - 1.8)	< 0.001
Streptococcus sanguis	3.88 ± 0.08 (3.8 - 4.0)	2.00 ± 0.12 (1.9 - 2.2)	2.96 ± 0.11 (2.8 - 3.1)	2.32 ± 0.07 (2.2 - 2.4)	1.44 ± 0.11 (1.3 - 1.6)	3.38 ± 0.08 (3.3 - 3.5)	< 0.001

[a]Values are expressed as mean ± SD (minimum-maximum)

Table 2. Comparison of Mean Diameter of the Grown Colonies (mm) in Different Mediums Between the Two Types of Bacteria (N = 5)[a]

Variables	*Streptococcus mutans*	*Streptococcus sanguis*	P Value
Kent cigarettes	1.52 ± 0.08	3.88 ± 0.08	< 0.001
Winston cigarettes	1.88 ± 0.08	2.00 ± 0.12	0.108
Ultralight Winston cigarettes	1.40 ± 0.07	2.96 ± 0.11	< 0.001
Hookah	1.5 ± 0.07	2.32 ± 0.07	< 0.001
Air	0.94 ± 0.11	1.44 ± 0.11	< 0.001
Carbon dioxide	1.7 ± 0.07	3.38 ± 0.08	< 0.001

[a]Values are expressed as mean ± SD.

ans to reduce its use.

The present study had a major strength that was the method of smoke collection, which was similar to the way a human smokes a cigarette or hookah. In addition, these smokes were compared with pure CO_2 to study the effect of CO_2, as well. Yet, the present study had some limitations, including the fact that the bacterial growth was measured by the colonies' diameter, while cell count could give more detailed results. In addition, the pure concentration of nicotine, carbon monoxide, and other metabolites could not be measured, as they might alter, although we chose 5 plates for synchronizing any difference among the collected smokes in each group.

The present study forms an enhanced internal validity of results due to the exclusion of extraneous variables. However, oral cavity, especially the microbial biofilm, harbors various microbial species and hence numerous bacterial interactions exist, any of which may be affected by cigarette smoke, which limits the external validity of the results of present study. We believe that future studies are needed to address the issue in vivo to shed more light on the subject.

In conclusion, the results of the present study showed overgrowth of *S. mutans* and *S. sanguis* by carbon dioxide, Winston, Kent, Ultralight Winston, and fruit tobacco, compared to ordinary air, while the growth of *S. sanguis* was statistically greater than *S. mutans* in all groups, except in the Winston group. According to the results of the present study, in line with the literature, it is suggested that health policy makers implement measures to decrease the rate of smoking cigarettes and hookah, due to their significant increase in the bacterial overgrowth of the oral cavity and their role in periodontal diseases.

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Footnotes

Authors' Contribution: Farimah Sardari and Mohadese Shakerian were responsible for the experimental design of the study; Leili Alibafghi and Reza Bahramabadi were responsible for the execution techniques and microbiology examination; Farimah Sardari and Reza Bahramabadi were responsible for the statistical analysis. All authors reviewed and contributed to the writing of this manuscript.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Nguyen KH, Marshall L, Brown S, Neff L. State-Specific Prevalence of Current Cigarette Smoking and Smokeless Tobacco Use Among Adults - United States, 2014. *MMWR Morb Mortal Wkly Rep*. 2016;**65**(39):1045–51. doi: 10.15585/mmwr.mm6539a1. [PubMed: 27711031].

2. Warren GW, Alberg AJ, Kraft AS, Cummings KM. The 2014 Surgeon General's report: "The health consequences of smoking–50 years of progress": a paradigm shift in cancer care. *Cancer*. 2014;**120**(13):1914–6. doi: 10.1002/cncr.28695. [PubMed: 24687615]. [PubMed Central: PMC5928784].
3. Meysamie A, Ghaletaki R, Haghazali M, Asgari F, Rashidi A, Khalilzadeh O, et al. Pattern of tobacco use among the Iranian adult population: results of the national Survey of Risk Factors of Non-Communicable Diseases (SuRFNCD-2007). *Tob Control*. 2010;**19**(2):125–8. doi: 10.1136/tc.2009.030759. [PubMed: 20008159]. [PubMed Central: PMC2989156].
4. Wu F, Chen Y, Parvez F, Segers S, Argos M, Islam T, et al. A prospective study of tobacco smoking and mortality in Bangladesh. *PLoS One*. 2013;**8**(3). e58516. doi: 10.1371/journal.pone.0058516. [PubMed: 23505526]. [PubMed Central: PMC3594295].
5. Centers for Disease Control and Prevention . *National Center for Chronic Disease P, Health P, Office on S, Health. Publications and Reports of the Surgeon General. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*. Atlanta, GA: Centers for Disease Control and Prevention (US); 2010.
6. Bartal M. Health effects of tobacco use and exposure. *Monaldi Arch Chest Dis*. 2001;**56**(6):545–54. [PubMed: 11980288].
7. Paoletti L, Jardin B, Carpenter MJ, Cummings KM, Silvestri GA. Current status of tobacco policy and control. *J Thorac Imaging*. 2012;**27**(4):213–9. doi: 10.1097/RTI.0b013e3182518673. [PubMed: 22847588]. [PubMed Central: PMC3409436].
8. World Health Organization . *WHO global report on trends in prevalence of tobacco smoking*. 2015.
9. Ambrose BK, Rostron BL, Johnson SE, Portnoy DB, Apelberg BJ, Kaufman AR, et al. Perceptions of the relative harm of cigarettes and e-cigarettes among U.S. youth. *Am J Prev Med*. 2014;**47**(2 Suppl 1):S53–60. doi: 10.1016/j.amepre.2014.04.016. [PubMed: 25044196]. [PubMed Central: PMC4642861].
10. Eissenberg T, Shihadeh A. Waterpipe tobacco and cigarette smoking: direct comparison of toxicant exposure. *Am J Prev Med*. 2009;**37**(6):518–23. doi: 10.1016/j.amepre.2009.07.014. [PubMed: 19944918]. [PubMed Central: PMC2805076].
11. Arcavi L, Benowitz NL. Cigarette smoking and infection. *Arch Intern Med*. 2004;**164**(20):2206–16. doi: 10.1001/archinte.164.20.2206. [PubMed: 15534156].
12. Bergstrom J. Tobacco smoking and subgingival dental calculus. *J Clin Periodontol*. 2005;**32**(1):81–8. doi: 10.1111/j.1600-051X.2004.00638.x. [PubMed: 15642063].
13. Aligne CA, Moss ME, Auinger P, Weitzman M. Association of pediatric dental caries with passive smoking. *JAMA*. 2003;**289**(10):1258–64. doi: 10.1001/jama.289.10.1258. [PubMed: 12633187].
14. Brook I. Effects of exposure to smoking on the microbial flora of children and their parents. *Int J Pediatr Otorhinolaryngol*. 2010;**74**(5):447–50. doi: 10.1016/j.ijporl.2010.01.006. [PubMed: 20129680].
15. Kreth J, Merritt J, Qi F. Bacterial and host interactions of oral streptococci. *DNA Cell Biol*. 2009;**28**(8):397–403. doi: 10.1089/dna.2009.0868. [PubMed: 19435424]. [PubMed Central: PMC2903342].
16. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. *J Clin Microbiol*. 2008;**46**(4):1407–17. doi: 10.1128/JCM.01410-07. [PubMed: 18216213]. [PubMed Central: PMC2292933].
17. Ertel A, Eng R, Smith SM. The differential effect of cigarette smoke on the growth of bacteria found in humans. *Chest*. 1991;**100**(3):628–30. doi: 10.1378/chest.100.3.628. [PubMed: 1889244].
18. Qvarfordt I, Riise GC, Andersson BA, Larsson S. Lower airway bacterial colonization in asymptomatic smokers and smokers with chronic bronchitis and recurrent exacerbations. *Respir Med*. 2000;**94**(9):881–7. doi: 10.1053/rmed.2000.0857. [PubMed: 11001080].
19. Zonuz AT, Rahmati A, Mortazavi H, Khashabi E, Farahani RM. Effect of cigarette smoke exposure on the growth of Streptococcus mutans and Streptococcus sanguis: an in vitro study. *Nicotine Tob Res*. 2008;**10**(1):63–7. doi: 10.1080/14622200701705035. [PubMed: 18188746].
20. Li M, Huang R, Zhou X, Zhang K, Zheng X, Gregory RL. Effect of nicotine on dual-species biofilms of Streptococcus mutans and Streptococcus sanguinis. *FEMS Microbiol Lett*. 2014;**350**(2):125–32. doi: 10.1111/1574-6968.12317. [PubMed: 24164376].
21. Cogo K, Montan MF, Bergamaschi Cde C, D. Andrade E , Rosalen PL, Groppo FC. In vitro evaluation of the effect of nicotine, cotinine, and caffeine on oral microorganisms. *Can J Microbiol*. 2008;**54**(6):501–8. doi: 10.1139/w08-032. [PubMed: 18535638].
22. Ebrahimi H, Bazargani A, Shahidi SP, Karimi F, Ansarifard E. The comparison of the In vitro effects of cigar and cigarette smoke on the growth of Streptococcus mutans and Streptococcus sanguis. *Elixir Human Physio*. 2013;**54**:12625–30.
23. Brook I, Gober AE. Effect of smoking cessation on the microbial flora. *Arch Otolaryngol Head Neck Surg*. 2007;**133**(2):135–8. doi: 10.1001/archotol.133.2.135. [PubMed: 17309981].
24. Bibars AR, Obeidat SR, Khader Y, Mahasneh AM, Khabour OF. The Effect of Waterpipe Smoking on Periodontal Health. *Oral Health Prev Dent*. 2015;**13**(3):253–9. doi: 10.3290/j.ohpd.a32671. [PubMed: 25197731].
25. Javed F, Al-Kheraif AA, Rahman I, Millan-Luongo LT, Feng C, Yunker M, et al. Comparison of Clinical and Radiographic Periodontal Status Between Habitual Water-Pipe Smokers and Cigarette Smokers. *J Periodontol*. 2016;**87**(2):142–7. doi: 10.1902/jop.2015.150235. [PubMed: 26430928].
26. Akl EA, Gaddam S, Gunukula SK, Honeine R, Jaoude PA, Irani J. The effects of waterpipe tobacco smoking on health outcomes: a systematic review. *Int J Epidemiol*. 2010;**39**(3):834–57. doi: 10.1093/ije/dyq002. [PubMed: 20207606].
27. Sabahy AR, Divsalar K, Bahreinifar S, Marzban M, Nakhaee N. Waterpipe tobacco use among Iranian university students: correlates and perceived reasons for use. *Int J Tuberc Lung Dis*. 2011;**15**(6):844–7. doi: 10.5588/ijtld.10.0744. [PubMed: 21575309].
28. Mirahmadizadeh A, Nakhaee N. Prevalence of waterpipe smoking among rural pregnant women in Southern Iran. *Med Princ Pract*. 2008;**17**(6):435–9. doi: 10.1159/000151563. [PubMed: 18836270].