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Association of tobacco habits with dental caries and *Streptococcus mutans* count

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Abstract
Background and aim: The role of tobacco as a risk factor for dental caries is yet to be established firmly. The present study was undertaken to evaluate the association of tobacco habits with dental caries and *Streptococcus mutans* count
Materials and Methods: A cross sectional, analytical study was conducted among 345 subjects aged 18-65 years, which were divided into 3 groups as smokers, smokeless tobacco chewers and non-tobacco users. Data regarding tobacco use was collected using a questionnaire. Caries status was assessed using WHO Oral Health Assessment Form for Adults-2013. Unstimulated whole saliva sample was collected for observing *Streptococcus mutans* colonies.
Statistical Analysis: ANOVA (Analysis of variance) and the post hoc test using Fisher's least significant difference (LSD) test. Pearson's correlation was done to assess the degree of correlation of DMFT with tobacco use and *Streptococcus mutans*.
Results: Smokeless tobacco users had highest mean DMFT (9.06±2.37) followed by smokers and non-tobacco users. The tobacco users with high *Streptococcus mutans* count had higher caries prevalence as compared to non-tobacco users. A statistically significant relation was found between caries prevalence and tobacco use.
Conclusion: The results revealed increased number of *Streptococcus mutans* count in smokeless tobacco users followed by smokers and non-smokers which may indicate increased caries susceptibility among tobacco users.

Keywords: *Streptococcus mutans*, Caries, tobacco habit

1. Introduction
Tobacco use imposes a massive and ever rising burden for public health globally.
The Portuguese introduced tobacco plant, *Nicotiana tabacum* in India during 1600AD during the Mughal era [1]. Initially the tobacco plant was cultivated for medicinal purposes like treating pain, poisonous bites, ulcers [2]. Many social, economic and political factors have contributed to the global spread of tobacco consumption.
About 35-40% of tobacco consumption in India is in smokeless forms, mostly of the species *Nicotiana rustica*, while most smoking tobacco is *Nicotina tabacum* [3].
Tobacco use imposes an ever rising health as well as economic burden. According to the World Health Organization (WHO), nearly 6 million deaths occur every year due to tobacco use, which may increase to 8 million deaths a year by 2030 [4]. The current burden of tobacco use in India includes 1 million deaths per year and billions of rupees of direct attributable health costs [3, 5]. Although the prevalence of tobacco use has reduced from 34.6% in GATS-1 (2009-10) to 28.6% in GATS-2 (2016-17) [6], it still continues to levy a large health burden.
In the oral cavity, saliva is the first biological fluid to be affected by the numerous toxic compositions of various tobacco forms, yet the means by which tobacco modifies the caries process in the mouth is still unclear [7]. Very few studies have explored the association between tobacco consumption and colony count of *Streptococcus mutans*. Some studies have shown an increased number of *Streptococcus mutans* colony count in tobacco chewers [8]. However, the association between smoking and *S. mutans* growth is controversial. Some research has revealed a positive relationship between smoking and *S. mutans* growth as tobacco has been shown to enhance the adhesion of *S. mutans* to the acquired pellicle [9].
Dental caries has a multifactorial etiology. There is a suggestive relation between tobacco use and dental caries incidence.

Some studies suggest increased mean DMFT among tobacco chewers followed by smokers. There are only a few number of studies that have investigated the relationship between the prevalence of dental caries among tobacco users consuming different forms of tobacco [10-13].

The present study was conducted to explore the association of tobacco habits with dental caries prevalence and subsequently *Streptococcus mutans* count among tobacco users and non-users.

2. Materials and methods

A cross sectional, analytical study was conducted among the patients reporting to the outpatient Department, Krishnadevaraya College of Dental Sciences and Hospital, Bangalore. The study participants who fulfilled all the study criteria were selected.

Inclusion Criteria

1. Adults, aged 18-65 years who currently smoke or chew tobacco.
2. Non-tobacco users, aged 18-65 years.
3. Patients with at least ten natural teeth.

Exclusion Criteria

1. Subjects not willing to participate in the study.
2. Dental fluorosis
3. Patients suffering from systemic diseases like diabetes mellitus, xerostomia, renal disease, radiotherapy.

Based on previous study [14] a sample of 345 subjects was selected using formula,

$$N = \frac{2\sigma^2 [Z_{1-\alpha/2} + Z_{1-\beta}]^2}{\mu_d^2}$$

with a power of 80% and Confidence interval = 95%. A simple random sampling was performed and the selected subjects were divided into 3 groups based on their tobacco habit as smokers, smokeless tobacco users and non-tobacco users. The survey period extended from the month of November 2017 to May 2018. Interviewing and examination of the study subjects was followed by saliva collection. The institutional ethical review board approved the study protocol. Written informed consent was obtained from each study subject after explaining the purpose of the study.

2.1 Caries assessment

Decayed, Missing and Filled (DMFT) teeth status as well as decayed, missing, filled surfaces (DMFS) was calculated from WHO Oral Health Assessment Form for Adults 2013. The CPI probe was used to confirm visual evidence of caries on the tooth surface. Caries was recorded to be present according to WHO criteria.

2.2 Microbial examination of Saliva Sample

After oral examination, study participants were asked to swish their mouth with water for one minute. Then the unstimulated whole saliva sample was collected in sterile screw capped plastic containers. The saliva sample was vortexed in the vortex mixer for one minute. 100µl of collected saliva was transferred to 10ml of sterile BHI (Brain heart infusion – HiMedia, Mumbai) broth and again vortexed for 30 seconds. 100µl of this dilution was inoculated on a sterile Mitis Salivarius Bacitracin agar (HiMedia, Mumbai) using L shaped spreader (Tarsons, Kolkata). The inoculated culture plates were incubated at 37 ° Celsius in a candle extinction jar for 24 hours. Small convex deep blue colonies on the agar plates were further studied by gram stain and identification tests. *Streptococcus mutans* were identified by gram stain morphology as gram positive cocci occurring in chains. The colonies were counted using a digital colony counter and the Colony Forming Unit per ml (CFU/ml) of the saliva sample was calculated.

2.3 Statistical Analysis

Caries and *Streptococcus mutans* count were dependent variables whereas tobacco use was independent variable. The collected data on tobacco habits, dental caries and *Streptococcus mutans* was subjected to statistical analysis by using ANOVA (Analysis of variance) and the post hoc test using Fisher's least significant difference (LSD) test. Pearson's correlation was done to assess the degree of correlation of DMFT with tobacco use and *Streptococcus mutans*. The level of significance was set at 5% ($P \leq 0.05$*).

3. Results

The study comprised of 345 subjects who were divided into 3 groups as smokers, smokeless tobacco users and non-tobacco users. There were 327 males and 18 females in the study sample.

Smokeless tobacco users had highest mean DMFT (9.06±2.37) as well as mean DMFS (27.95±21.37) followed by smokers (5.98±2.21) and non-tobacco users (3.45±1.63) but this difference was found to be statistically not significant (Table 2). A statistically significant difference was seen in relation to missing teeth with smokeless tobacco users having highest mean number of missing teeth (3.03±1.43). Table 3 shows that almost all smokers and smokeless tobacco users had high *Streptococcus mutans* count (>10^5CFU/ml). Entire group of non-tobacco users had low *Streptococcus mutans* count (<10^5 CFU/ml). This finding may suggest that tobacco use enhances the growth of *Streptococcus mutans*. Data collected regarding oral hygiene practices showed that brushing frequency of once daily was seen in most of the study participants with only a few smokers (1.74%) brushing their teeth twice daily as compared to 6.08% among non-tobacco users (Table 4). Table 5 shows that a statistically significant moderate correlation was found between dental caries and duration of smokeless tobacco use (years). A moderate positive correlation was obtained between tobacco use (smoked/smokeless tobacco) and DMFT where as a strong correlation was obtained between tobacco use and *Streptococcus mutans*. A positive correlation of caries experience with tobacco use suggests that tobacco use has an effect on caries prevalence as well as *Streptococcus mutans* count (Table 6).

Table 1: Tobacco use (type, frequency and duration) among study groups

		Smokers (N=115)	Smokeless (N=115)	Nontobacco users (N=115)
Age group (yr)	18-24	1.74%	0%	28.70%
	25-34	3.47%	6.09%	39.13%
	35-44	77.39%	32.17%	25.21%
	45-54	11.30%	32.17%	5.21%
	55-65	6.08%	29.56%	1.74%
Type of tobacco used	Cigarette	82.6%	0	0
	Bidi	17.4%	0	0
	Betel nut	0	0.86%	0
	Betel nut with leaf	0	20.8%	0
	Guthka	0	27.8%	0
	Hans	0	50.4%	0
Frequency	1-5	54.7%	80.2%	0
	6-10	31.8%	10.2%	0
	>10	13.5%	9.6%	0
Duration	<1year	10.4%	3.48%	0
	2-5years	33.04%	18.26%	0
	6-10years	44.34%	20.87%	0
	>10 years	12.17%	57.39%	0

Table 2: Caries status among the study groups

	Smokers (N=115)	Smokeless tobacco users (N=115)	Non-tobacco users (N=115)	P Value
DT	3.56±0.78	4.74±0.69	3.27±1.25	0.07
MT	1.69±1.07	3.03±1.43	1.08±1.09	0.01*
FT	1.22±1.29	1.28±1.40	0.95±1.23	0.27
DMFT	5.98±2.21	9.06±2.37	3.45±1.63	0.53
DMFS	13.95±7.06	27.95±21.37	12.65±8.18	0.082

Table 3: *Streptococcus mutans* count among the study groups

Streptococcus mutans (CFU/ML)	Smokers (N=115)		Smokeless tobacco users (N=115)		Non-tobacco users (N=115)	
Low	4	3.5%	0	0%	115	100%
High	111	96.5%	115	100%	0	0%
Total	115	100%	115	100%	115	100%

Table 4: Distribution of study groups according to brushing frequency

Brushing frequency	Smokers (N=115)		Smokeless tobacco users (N=115)		Non-tobacco users (N=115)	
Once	113	98.26%	115	100%	108	93.92%
Twice	02	1.74%	0	0%	7	6.08%
After every meal	0	0%	0	0%	0	0%
Never	0	0%	0	0%	0	0%
Total	115	100%	115	100%	115	100%

Table 5: Correlation of dental caries with duration of tobacco use

		Pearson's Correlation Coefficient (R)	P-Value
DMFT	Duration of smoking (years)	0.088	0.104
	Duration of smokeless tobacco use (years)	0.668	0.000*

Table 6: Correlation between tobacco use and caries prevalence, *Streptococcus mutans* count

		Pearson's Correlation Coefficient (R)	P Value
Tobacco use	DMFT	0.739	0.000*
	Streptococcus mutans	0.859	0.000*

4. Discussion

In spite of the adverse health and financial implications of tobacco use, it still remains one of the leading causes of preventable deaths in the world. Due to work pressure and stressful lifestyle, people are getting inclined towards tobacco use. Tobacco use is strongly implicated in the development of periodontal disease but its implication on development of caries is yet to be established [4, 5]. There arises a need to see if there is any association between tobacco habits and caries pattern. The present study is one of the few to assess the effects of both smoking and smokeless tobacco consumption on dental caries and *Streptococcus mutans* count.

In the present study, the mean age of smokers, smokeless tobacco users and non-users was 35.64±08.17 years, 32±03.45 years and 30±06.41 years respectively. Information collected regarding age of habit initiation among smokers revealed that minimum age of initiation of tobacco habit was 23.4 years which is similar to one reported by Jindal SK *et al.* [15] and Aguilar-Zinser V *et al.* [16] Early initiation of tobacco habits is generally a result of easy availability of tobacco products and peer influence.

The finding in the present study shows that majority of smokers were cigarette smokers, followed by bidi smokers. Similar results were found in the previous studies conducted by Jabeen S *et al*. [17] and Mankar RB [18]. Rapid income growth over the last decade has most likely contributed to the shift in smoking from the less-expensive bidis to cigarettes. Among smokeless tobacco users, there was predominance of hans use (local smokeless tobacco form) followed by guthka use. Despite the ban, the unrestricted smokeless tobacco consumption can be attributed to easy availability of attractive, conveniently packed sachets that result in hassle free chewing.

The duration of tobacco smoking shows that majority (44.34%) smoked for 6-10 years. Mean years of tobacco smoking was found to be 6.19±3.47 years. Bashiru BO *et al*. [19] and Katuri KK *et al*. [5] reported similar findings.

Highest mean DMFT was recorded among smokeless tobacco users (9.06±2.37) followed by smokers and non-tobacco users. Similar findings were observed by Vellappally S *et al*. [10]. The mean number of decayed teeth was highest in tobacco chewers followed by smokers and non-tobacco users but the results were not statistically significant. Smokeless tobacco users showed statistically significant higher mean number of missing teeth as compared to smokers and non-tobacco users, which is similar to previous studies done by Amjad F *et al*. [20] and Tomar SL *et al*. [21]. Increased caries incidence in smokeless tobacco users may be possibly explained by the fact that smokeless tobacco contains high amounts of caries promoting sugars like sucrose, fructose, which stimulate the growth of cariogenic bacteria.[22] Whereas, the decreased buffering effect, possible lower pH of smoker's saliva, consumption of mint flavoured candies to mask the smell of tobacco and higher number of Lactobacilli and *Streptococcus mutans* group may indicate an increased susceptibility to caries in smokers [23].

High *Streptococcus mutans* (>10^5 CFU/ml) was found among smokers and smokeless tobacco users where as low *Streptococcus mutans* count (<10^5 CFU/ml) was found among non-tobacco users. Similar results were obtained in study conducted by Heintze U [24] in which about 40% of the smokers had > 10^6 *Streptococcus mutans* CFU/ml, which was more than twice that of non-smokers and Sellappa S *et al*. [8] reported increased number of *Streptococcus mutans* in smokers and chewers (56.6% and 63.3%) respectively. Similarly, *in vitro* studies conducted by Lindemeyer RG *et al*. [25], Li M *et al*. [26] and Arun J *et al*. [27] reported enhanced *Streptococcus mutans* growth in the presence of tobacco extracts.

In the present study, salivary levels of *S. mutans* were related to their DMFT scores. It was found that overall DMFT was lower when the levels of *S. mutans* was less and increases with the increase in bacterial count. Similar results were reported by Pannu P *et al*. [28] in which the mean DMFT recorded using Moller's index, was found to be maximum (5.89 4.78) in individuals with high *S. mutans.* Smokeless tobacco users with high *Streptococcus mutans* count had higher mean DMFT whereas non-tobacco users with low *Streptococcus mutans* count had lower mean DMFT.

5. Conclusion

Within the parameters of this study, it was found that tobacco use exhibited quite a significant role on dental caries. A statistically significant positive correlation was seen between tobacco use and dental caries, *Streptococcus mutans* count suggestive of contributory effect of tobacco use on caries prevalence. Microbial analysis of saliva showed that there was a substantial increase in the microbial load of *Streptococcus mutans* among tobacco users which might increase the caries severity. However, a direct causal role of tobacco use in caries could not be established. There is a need for further longitudinal studies in a larger population to confirm the relationship of tobacco consumption with dental caries.

6. References

1. Reddy KS, Gupta PC. Report on Tobacco Control in India. Ministry of Health & Family Welfare, Government of India, 2004.
2. Charlton A. Medicinal uses of tobacco in history. J R Soc Med. 2004; 97(6):292-296.
3. McKay AJ, Patel RKK, Majeed A. strategies for tobacco control in India: a systematic review. PLoS One. 2015; 10(4).
4. Singh A, Ladusingh L. Prevalence and Determinants of Tobacco Use in India: Evidence from Recent Global Adult Tobacco Survey Data. PLoS One. 2014; 9(12).
5. Katuri KK, Alluri JK, Chintagunta C, Tadiboina N, Borugadda R, Loya M *et al*. Assessment of Periodontal Health Status in Smokers and Smokeless Tobacco Users: A Cross-Sectional Study. Journal of Clinical and Diagnostic Research. 2016; 10(10).
6. Fact sheet Global Adult Tobacco India (GATS-2). Accessed from: Ministry of Health and Family Welfare, Government of India, 2016-17.
7. Mujahid M, Anushree B, Shobha M. Salivary Levels of Cariogenic *Streptococcus* and *Lactobacillus* among Tobacco Abusers in Andhra Pradesh, India. RJPBCS. 2014; 5(6):521-530.
8. Sellappa S, Rajamanickam U, Selvaraj V, Khan MR, Vijayakumar S. Association of bacterial growth in the oral cavity between tobacco smokers and tobacco chewers. International Journal of Toxicological and Pharmacological Research. 2015; 7(4):228-231.
9. Campus G, Cagetti M, Senna A, Blasi G, Mascolo A, Demarchi P *et al*. Does Smoking Increase Risk for Caries? A Cross-Sectional Study in an Italian Military Academy. Caries Res. 2011; 45:40-46.
10. Vellappally S, Jacob V, Smejkalova J, Shriharsha P, Kumar V, Fiala Z. Tobacco habits and oral health status in selected Indian population. Cent Eur J Public Health. 2008; 16(2):77-84.
11. Axelsson P, Paulander J, Lindhe J. Relationship between smoking and dental status in 35–, 50–, 65–, and 75-year-old individuals. J Clin Periodontol. 1998; 25(4):97-305.
12. Bruno-Ambrosius K, Swanholm G, Twetman S. Eating habits, smoking and tooth brushing in relation to dental caries: a 3-year study in Swedish female teenagers. Int J Paediatr Dent. 2005; 15(3):190-6.
13. Ide R, Mizoue T, Ueno K, Fujino Y, Yoshimura T. Relationship between cigarette smoking and oral health status. Sangyo Eiseigaku Zasshi. 2002; 44(1):6-11.
14. Drope J, Schluger N, Cahn Z, Drope J, Hamill S, Islami F *et al*. The Tobacco Atlas American Cancer Society. 6th edition. 2018.
15. Jindal SK, Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D *et al*. Tobacco Smoking in India: Prevalence, Quit-rates and Respiratory Morbidity. Indian J Chest Dis Allied Sci. 2006; 48(1):37-42.
16. Aguilar-Zinser V, Irigoyen ME, Rivera G, Maupomé G, Sánchez-Pérez L, Velázquez C. Cigarette Smoking and

Dental Caries among Professional Truck Drivers in Mexico. Caries Res. 2008; 42:255-262.
17. Jabeen S, Manni UJA, Shakil SS. Oral health status among tobacco users in the selected rural population. Bangladesh Med J. 2014; 43(2).
18. Mankar RB. Association between a periodontal disease, smokers & smokeless tobacco in Rajnandgaon population. International Journal of Applied Dental Sciences. 2018; 4(3):161-163.
19. Bashiru BO, Udo UA. Cigarette smoking and Awareness of Oral Health Problems of Tobacco use among Students at the University of Port Harcourt, South-South Nigeria. World Journal of Dentistry. 2014; 5(4):209-212.
20. Amjad F, Ali S, Bhatti Mud, Chaudhry AH. Effects of tobacco chewing on oral health status of patients visiting University College of dentistry, Lahore. Pakistan Oral & Dental Journal. 2012; 32(3).
21. Tomar SL, Winn DM. Chewing tobacco use and dental caries among US men. JADA. 1999; 130:1601-10.
22. Vikneshan M, Ankola AV, Hiremath A, Hebbal M, Suganya M. Smokeless tobacco and its adverse effects on oral cavity- an overview. Annals of Dental Specialty 2014; 2(2).
23. Winn DM. Tobacco Use and Oral Disease. Journal of Dental Education. 2001; 65(4).
24. Heintze ULF. Secretion rate, buffer effect and number of lactobacilli and *Streptococcus mutans* of whole saliva of cigarette smokers and nonsmokers. Department of Cariology, School of Dentistry. 1984; S-214 21.
25. Lindemeyer RG, Baum RH, Hsu SC, Going RE. *In vitro* effect of tobacco on the growth of oral cariogenic streptococci. JADA. 1981; 103(5):719-22.
26. Li M, Huang R, Zhou X, Zhang K, Zheng X, Gregory R. Effect of nicotine on dual-species biofilms of *Streptococcus mutans* and *Streptococcus sanguinis*. FEMS Microbiol Lett. 2014; 350(2):125-32.
27. Arun J, Srikant N, Suman E, Shenoy A, Baliga S. Effect of tobacco extract on *Streptococcus mutans* : possible role in modulating carcinogenesis. Asian J Pharm Clin Res. 2018; 11(7):398-401.
28. Pannu P, Ghambhir R, Sujlana A. Correlation between the salivary *Streptococcus mutans* levels and dental caries experience in adult population of Chandigarh, India. Eur J Dent. 2013; 7(2):191-195.