Research Article

Influence of Salivary Flow Rate and Salivary pH on Dental Caries in Smokers and Non Smokers-A Cross Sectional Study

Jayachandra MY*

Department of Dental Surgery, Raja Rajeswari Dental College and Hospital, Bangalore, India

Corresponding author:
Jayachandra MY,
Department of Dental Surgery,
Raja Rajeswari Dental College and Hospital,
Bangalore, India,
E-mail: jaichandra.my@gmail.com
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Abstract

Background and objectives: Saliva is detrimental to both general and oral health. Though the effects of smoking on oral mucosa had been demonstrated, independent variables of saliva such as salivary low, pH, and combined effect on the dental caries experience is unknown and worthy of investigation. **Materials and methods:** A cross-sectional study was conducted to determine the Salivary Flow Rate (SFR) and salivary pH in smokers and non-smokers with and without dental caries. The study was conducted at Rajarajeswari dental college and hospital, Bangalore on 120 patients categorized into four groups, smokers with dental caries (30 subjects), smokers without dental caries (30 subjects), non-smokers with dental caries (30 subjects) and non-smokers without dental caries (30 subjects) unstimulated saliva was collected from the subjects and salivary low rate and salivary pH was measured. **Results**: The mean salivary low rate (mean salivary low–0.415 ml/min) and salivary pH (mean pH 5.4) was significantly lower among smokers than non-smokers which was statistically significant ($p<0.001$). A statistically significant negative correlation of salivary pH among smokers with dental caries was found. **Conclusion:** Salivary low rate and pH was reduced among smokers than non-smokers. In both smokers and non-smokers, subjects with dental caries had lower salivary low rate, pH.

Keywords: Salivary flow rate; Salivary pH; Smoking; Dental caries; Non Smokers

Introduction

Saliva is a complex, versatile and important body fluid secreted by three pair of major salivary glands [1]. Saliva constitutes a first line of defense against oxidative stress and has protective effects against toxins and antioxidants [2]. Many studies on saliva report the physicochemical properties of saliva (flow rate, buffer capacity, and pH) or the concentration of components of the saliva with antimicrobial activities [3]. Dental caries is one of the most common, but rarely life threatening disease that ends up in the destruction of hard dental tissue. Imbalances in levels of free radicals, reactive oxygen species and antioxidants in saliva play an important role in the onset and development of dental caries [4-6]. One factor not recognized as a risk factor for caries, in the CAMBRA model, is cigarette smoking. Saliva is the first biological fluid that is exposed to cigarette smoke, which spreads to all parts of the oral cavity and therefore, the taste receptors, a primary receptor site for salivary secretion are constantly exposed to many toxic compounds responsible for structural and functional changes in saliva [6,7]. Studies examined the relationship between early childhood caries and parental smoking and concluded there is an association between environmental tobacco smoke and risk of caries among children and adolescents [8,9]. The literature is lacking studies in India that examine smoking in relation to caries using biological dependant variables. Thus, an attempt is made in this study to evaluate the influence of salivary

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parameters such as salivary flow rate, pH on dental caries in smokers and non-smokers attending the outpatient department of Rajarajeswari dental college and Rajarajeswari medical college and hospital, in Bangalore.

Materials and Methods

Study design

An observational, cross-sectional, institution based study design was conducted for a period of 8 months to find out the correlation between salivary pH and flow with dental caries in smokers and non-smokers.

Study population selection

The present study was done at Rajarajeswari dental college and Rajarajeswari medical college and hospital. Bangalore. India. Subjects were selected from the department of oral medicine and radiology; and Outpatient Department (OPD) of Rajarajeswari medical college if they met the eligibility criteria.

Inclusion criteria

- Those subjects aged 20-40 years and attending the outpatient department of Rajarajeswari dental college and Rajarajeswari medical college and hospital.
- Only current daily smokers (defined as, A daily smoker is a person, who smokes any tobacco product at least once a day as per WHO criteria 8) for at least 1 year with and without dental caries, will be included as smokers in the present study.
- Only never smokers (defined as, A never-smoker is a person who has never smoked at all WHO criteria) with and without dental caries, will be included as non-smokers in present study.

Exclusion criteria

- Medically compromised patients or subjects with a history of conditions/medications/therapy that alter the salivary parameters are excluded from the study.
- Subjects who are fasting and have nausea.
- Subjects having periodontal disease and poor oral hygiene.
- Those who have not given informed consent.
- Subjects who are concurrent users of smokeless tobacco in any form.
- Subjects should not have consumed any medication for at least 15 days preceding saliva collection.

Sample size determination

The significance level (α)=5%.

Power of the study=0.98.

$N=2x(Z\alpha(S.d))/d^2=30$ subjects per group.

Where;

Zα-Normal value for the probability level=2 at 5% level.

N-Sample size.

S.d-Standard deviation.

d-Difference in the means.

The study comprised of 120 participants who met the eligibility criteria and provided written informed consent, belonging to age group 20-40 years, in four groups,

- **Group 1:** Smokers with dental caries (30 subjects).
- **Group 2:** Smokers without dental caries (30 subjects).
- **Group 3:** Non-smokers with dental caries (30 subjects).
- **Group 4:** Non-smokers without dental caries (30 subjects).

The investigator was calibrated at the department of public health dentistry, Rajarajeswari dental college and hospital under the guidance of the professor in order to limit the examiner variability. The calibration session for minimizing the inter examiner variability (phase I calibration) was done first followed by the calibration for minimizing the intra examiner variability (phase II calibration). The kappa co-efficient values for inter examiner and intra examiner variability with respect DMFT index was 0.89.

Examination procedure

Study was carried out using a specific preformat consisting of two parts.

First part consisted of general information regarding the patient's demographic profile, medical history, history of smoking, and oral hygiene practices and personal habits which were recorded through an interview designed in English using a structured proforma. The second part comprised of clinical examination and collection of salivar. Caries experience was recorded using DMFT index given by Henry Klein, Carrole Palmer and Knutson in 1997 modification (decayed missing and filled tooth index) according to WHO criteria. Examination was carried out with diagnostic instruments and under adequate illumination [10-13].

Saliva collection

Unstimulated saliva was collected from each subject between 8-10 a.m. to avoid circadian variations. Patients are instructed not to drink, eat, smoke or put anything into their mouths for 90 minutes before the collection time. The dentist or designated staff member collects the saliva in a quiet environment, with the patient sitting in an upright position, head tilted forward and eyes open, with minimal body and orofacial movements. The patient is asked to swallow saliva first, then stay motionless and allow the saliva to accumulate in oral cavity for five minutes, after five minutes the patient is asked to void the mouth of saliva by expectorating into a graduated container. Patient was asked not to swallow or talk during the collection period.

Salivary analysis

GC saliva check buffer is a comprehensive kit, which was used to measure pH, flow and buffering capacity of saliva.

Estimation of salivary flow rate

After the collection of saliva in a graduated container, the total salivary volume was measured using the lower meniscus level container. The unstimulated salivary flow rate was estimated per minute by dividing the total value of the volume with 5 and flow rate is expressed in terms of milliliter per minute (ml/min).

Estimation of salivary pH

The sample of saliva unstimulated thus collected was used to estimate the pH using the colorimetric pH strips. The range of the pH strips were from 3-6 and 6.5.

Using a micropipette a sample of collected of unstimulated saliva was withdrawn and two to three drops of this is place in the pH strip and left for 2 minutes. The pH was estimated by the color change in the strip and the reading was immediately taken correlating with the chart provided along with the kit and corresponding pH value is recorded [14-16].

Statistical tests employed

Data was entered into excel spreadsheet and tested for normality assumption using Kolmogorov-Smirnov test. Proportions were compared using *Chi-square* test of significance. Mann-Whitney U test was used to find the variances between the groups. Spearman's correlation coefficients were calculated to determine correlation between the DMFT data and the salivary flow rate and salivary pH. In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance. Data analysis was carried out using Statistical Package for Social Science (SPSS ver 21) package [17-19].

Results

Distribution of study population

Table 1 shows the distribution of study population according to age.

Table 1: Distribution of study population according to age.

Smoking status		Age		Total	X^2 value	'p' value
		20-29 yrs	30-40 yrs			
Smoker	With caries	17	13	30	0.624	0.43
		58.60%	41.40%	100.00%		
	Without caries	14	16	30		
		48.30%	51.70%	100.00%		
	Total	31	29	60		
		53.40%	46.60%	100.00%		
Non smokers	With caries	14	16	30	0.648	0.421
		44.80%	55.20%	100.00%		
	Without caries	11	19	30		
		34.50%	65.50%	100.00%		
	Total	25	35	60		
		39.70%	60.30%	100.00%		

The total study population of 60, among smokers majority of the population were of age group 20-29 years *i.e.* 31 (53.4%). Among smokers (n=60) majority of the carious subjects (n=17, 58.9%) were of age group 20-29 years and among those without caries majority (n=16, 51.7%) were of the age group 30-40 years. Among non-smokers majority of the study population (n=35, 60.3%), was of the age group 30-40 years. Among non-smokers those with caries, majority 16 (55.2%) were of age group 30-40 years and those without caries 19 (65.5%) were also of the same age group.

Salivary pH

Table 2 shows the mean salivary pH among smokers and non-smokers with and without dental caries.

Table 2: Salivary pH among smokers and non-smokers.

Smoking status	Caries	N	Mean	SD	Median	Min.	Max.	Mann-Whitney U	'p' value
Smoker	With	30	5.469	0.252	5.4	5.2	6	100.5	<0.001
	Without	30	5.89	0.211	5.8	5.6	6.2		
Non smokers	With	30	6.217	0.277	6.2	5.8	6.6	18.5	<0.001
	Without	30	6.986	0.296	7	6.5	7.4		

Among smokers pH ranged from 5.2–6.2; the mean pH of smokers with caries was less (5.469) when compared to subjects without caries (5.890) and the results in smokers was highly significant with the obtained p value (<0.001).

Among non-smokers the ph ranged from 5.8-7.4 for both the groups. The mean pH was lesser in carious group (6.217) as compared to non-carious group (6.986) and the results were statistically significant with the obtained p-value (<0.001).

The pH among smokers was highly acidic when compared to non-smokers; in both the groups the subjects with caries had lower pH as compared to carious subjects.

Salivary flow rate

Table 3 shows the salivary flow rate in smoker and non-smokers with and without dental caries.

Table 3: Salivary flow among smokers and non-smoker study population with and without dental caries.

Smoking status	Caries	N	Mean	SD	Median	Min.	Max.	Mann-Whitney U	'p' value
Smoker	With	30	0.415	0.03	0.41	0.38	0.48	80	<0.001
	Without	30	0.486	0.046	0.49	0.41	0.58		
Non smokers	With	30	0.641	0.088	0.63	0.52	0.79	7	<0.001
	Without	30	1.008	0.15	1	0.7	1.3		

Among a total of 60 study population of smokers, the salivary flow ranged from 0.38 ml/min-0.58 ml/min with the mean salivary flow rate among smoking non carious subjects more (0.486 ml/min) as compared to carious subjects. The results were significant with the obtained p value (<0.001). Among a total of 60 study populations of non-smokers, the salivary flow ranged from 0.52 ml/min-1.30 ml/min with the mean salivary flow rate of among non-smoking, non-carious subjects (0.415 ml/min) more as compared to carious subjects. The results were significant with the obtained p value (<0.001). The salivary flow rate among smokers was less when compared to non-smokers and non-carious subjects in both the groups had a mean salivary flow greater than those with carious lesions [20].

Correlation of DMFT with salivary pH and flow rate

On comparing pH values among smokers and non-smokers, salivary pH values of the subjects in both the groups showed a negative correlation with DMFT, but among smokers (r=-0.450, n=30) pH was found to be statistically significant (p=0.014). DMFT scores increases with decrease in salivary pH among smokers. When salivary flow rate was correlated with DMFT scores among smokers and non-smokers there was no statistically significant correlation found. Thus in the present study unstimulated salivary flow had a positive correlation among smokers (r=0.180, p=0.351, n=30) and non-smokers (r=0.039, p=0.841, n=30), which was not significant. Thus in the present study unstimulated salivary flow had a positive correlation among smokers (r=0.180, p=0.351, n=30) and non-smokers (r=0.039, p=0.841, n=30), which was not significant.

Discussion

Saliva has an old history of study but its physiological importance has only been recognized recently. Nowadays, the saliva research field is rapidly advancing due to the use of novel approaches that include metabolomics, genomics, proteomics and bioinformatics. Saliva plays a critical role in oral homeostasis. Under resting conditions there is a slow flow of saliva which keeps the mouth moist and lubricates the mucous membrane. Dentists should be more aware of their patients' salivary function and include more disciplined preventive practices to deter the negative effects. In the present study contributing (medication, radiation therapy, systemic diseases) as well as protective (fluoride and xylitol) factor was over looked and studies on longitudinal scale are needed to determine the exact relationship of smoking on salivary parameters, oral and dental disease.

In our study we have chosen to measure unstimulated saliva, noninvasive and comfortable procedure, which favors its use in population studies. Saliva is the first biological fluid that is exposed to tobacco (smoked/smokeless form), which contains numerous toxic compositions responsible for structural and functional changes in saliva. The present study confirmed a statistically significant difference (p<0.001) in salivary parameters in smokers and non-smokers. There was significant difference found in Salivary Flow Rate (SFR) of smokers (range 5.2–6.2), being lesser than non-smokers

(5.8-7.4) in our study, which is in accordance with studies conducted by, Volekar, et al. in US; Rad M, et al. Iran. Kanwar A, et al., in India stated, salivary flow rate in smokers is probably due to the effect of nicotine on taste nerve apparatus. Khan GJ, et al., in Pakistan discovered that smoking increases the activity of salivary glands and, indeed, this observation has been made by everyone who begins smoking but in long run it decreases the salivary low rate. It has also been observed that some tolerance develops to the salutatory effects of smoking because habitual smokers do not salivate as do novice smokers in response to smoking and complain of dry mouth at young age. However, our results are comparable to studies that have shown smoking is one of the risk factors for reducing salivary flow rate and xerostomia by Nosratabadi, et al., in Iran and the reason for reduction in flow rate can be accounted due to toxic chemicals in cigarette smoke. In contrast, studies conducted by Athra, et al., in Baghdad, Chauhan S, et al., in India, Eliasson L, et al., in Sweden and one study done on passive smokers by Avsar A, et al., saw no difference in salivary flow rate among both the groups, which was not in agreement with the results of the present study.

Literature has explored a relationship between salivary flow rate and dental caries. Reduction in salivary flow rates has seen as an increase activity leading to dental caries. In the present study the salivary flow rate (5.4 ml/min–6.6ml/min) was significantly less in subjects with dental caries compared to those without dental caries, in both the groups which is in agreement with the studies conducted by Preethi BP, et al., in India. Rad M, et al., in Iran who observed caries was significantly higher in smokers than non-smokers. However a recent report indicated that exposure to tobacco smoke at the age of 4 months old was associated with a nearly 2 fold increased risk of developing dental caries, and the risk of caries was also increased among those who were exposed to household smoking by 1 to 1.5 fold 25. These studies once again proved the contact with cigarette smoke and accumulation of nicotine significantly increasing the risk of caries. Agluiar Z, et al., in US, Farsi, et al., in Saudi Arabia, Athra, et al., in Baghdad have shown that smokers have a significantly higher number of carious or repaired teeth than non-smokers, and heavy smokers are more affected than light smokers. Also it is reported that smokers have higher plaque rates, poorer oral hygiene habits and skills, fewer visits to dentists, and lesser overall health standards than nonsmokers. An *in-vivo* study on rats by Liu S, et al., concluded that there was an obvious increase in the incidence and severity of sulcal caries in the nicotine-treated group. Therefore, nicotine promoted the development of dental caries in rats challenged with *S. mutans*, these factors may be the reasons for the increased caries rate in smokers. In contrast, studies conducted by Ahmadi-M, et al., in Iran, Tulunoglu O, et al., in Turkey, showed no difference in salivary flow rate between caries free and caries active groups.

Use of tobacco in various forms and its interaction is known to cause abnormality in salivary pH. pH varies according to the SFR. Higher the SFR, higher the buffering capacity, thus higher the pH and vice versa. Alterations in salivary pH have a significant impact on oral and dental health and can be used for the diagnosis of a wide range of diseases. Saliva pH changes have been cited as variables for modifying caries risk. Eslami, et al.; Khan, et al., in Pakistan; Shubha, et al., in India Kusumaningrum, et al., from Thailand observed a lower salivary pH in smokers than in non-smokers. Study by Kanwar A, et al., in India showed salivary pH was found to be lower (mean pH 6.8) in tobacco smokers and tobacco chewers (mean pH 6.7) than in subjects with no such habits, but the difference was statistically insignificant. In the present study, salivary pH was different across the groups with lower pH in smokers than in non-smokers which was in agreement with the studies conducted. In contrast to studies conducted by Athra M, et al. in Baghdad; Chauhan, et al., in India and Volekar, et al., US showed no significant changes in salivary pH between smokers and non-smokers and were not in agreement to our study. Salivary pH and dental caries are interlinked as the pH reaches critical level (< 5.5) initiation of demineralization occurs. In the present study the pH of the caries group (mean pH 5.4) was less than the non-carious group (mean pH 5.8) irrespective of the smoking status. Our study findings are in agreement to the studies conducted by Mujahid M, et al., in India. Studies done by Ahmadi-Motamayel, et al., in Iran, Tulunoglu O, et al., in Turkey had contrasting result to our study and this may be due to individual and environmental variations.

Conclusion

In our study salivary flow rate, buffering capacity, pH was decreased among smokers than non-smokers. In both smokers and non-smokers, subjects with dental caries had lower salivary flow rate, pH. Tobacco use was clearly associated with risk of dental caries. This alteration of salivary flow rate and salivary pH can make the oral mucosa vulnerable to various oral and dental diseases. Qualitative and quantitative salivary assessment is a useful tool for screening many systemic and oral conditions. Clearly, saliva has profound effects on the oral cavity, but few dental practitioners don't bother to ask the necessary questions or make the necessary observations and/or measurements to determine whether there is any level of salivary gland hypo function in their patients.

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