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Original Article

Dentistry Section

Comparative Evaluation of Salivary Flow Rate in Smokers and Non Smokers: A Cross-sectional Study

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ABSTRACT

Introduction: Saliva is a complex and significant body fluid which is exceptionally fundamental for oral health and is the most effectively available fluid in the human body. Structural and function changes in saliva occur when it is exposed to cigarette smoke that contains several toxic components and is known to be one of the main risk factors for developing numerous oral conditions. There are conflicting reports about the correlation between cigarette smoking and mouth dryness. Additionally, there is paucity of studies to investigate the Salivary Flow Rate (SFR) in smokers in India.

Aim: To assess the salivary flow rate in smokers and non smokers and also to study the effect of duration and frequency of smoking habit on SFR.

Materials and Methods: This analytical cross-sectional study was conducted in which unstimulated whole saliva of 50 smokers and 50 non smokers visiting Outpatient Department (OPD) at Mahatma Gandhi Vidyamandir's, Karmaveer Bhausaheb Hiray Dental College and Hospital Nashik, Maharashtra, India, was from January 2019 to January 2020. Unstimulated SFR was measured and expressed in mL/minute using a graduated glass tube. Comparison of SFR was done among the smokers and non smokers using Unpaired t-test. One-way Analysis of Variance (ANOVA) test was used to compare the frequency and duration of smoking habit and SFR among smokers.

Results: There was a statistically significant reduction (p-value <0.0001) in the salivary flow rate of smokers in comparison to non smokers. It was also observed that there was reduction in salivary flow rate with increase in the duration (p-value <0.001) and frequency (p-value=0.012) of tobacco smoking habit.

Conclusion: Salivary flow rate is significantly reduced in smokers when compared to non smokers. The duration and frequency of smoking habit had a significant impact on the reduction in SFR. Reduced SFR has a high impact on oral health and may further lead to several oral complications. Therefore, measures should be taken by counseling the smokers to quit the habit and educating them about the ill-effects of xerostomia.

Keywords: Cigarette smoking, Saliva, Smoking, Tobacco smoking

INTRODUCTION

Saliva is a complex and significant body fluid which is exceptionally fundamental for oral health [1]. It is essential for maintaining the integrity of the oral mucosa, remineralization of teeth, aids in digestion, helps in taste sensation, has a wash-out effect to prevent demineralisation of teeth, maintains oral pH through its buffering capacity and helps in phonation. It is being employed to diagnose a wide scope of infections [2]. It is the most effectively available fluid in the human body which may subsequently serve as an easy tool for non invasive measurements of various body parameters [2]. Hence, saliva undertakes a critical role in maintaining the ecosystem and homeostasis of the oral cavity.

Salivary secretion is a complex process in which its composition as well as flow varies greatly under different circumstances [3]. It is a reflex function emerging from salivary centers that is dependent on afferent stimulation and involves complex assimilation from higher center [1]. Resting whole saliva is the mixture of secretions, which enters the mouth in the absence of exogenous stimuli [4]. Unstimulated salivation occurs as a result of autonomic stimulation. Daily secretion of saliva ranges from 0.75 to 1.5 L/day. Unstimulated Salivary Flow Rate (SFR) is approximately 0.3-0.5 mL/min [1,5,6] and the stimulated SFR can reach as high as 10 mL/ min [1].

Currently, one-third of the adult population is comprised of smokers [7]. The number of cigarette smokers is declining, but the frequency is increased in those who do smoke [7]. As of now, smoking is known to be one of the main risk factors for developing numerous oral conditions like tooth discoloration, mouth dryness, oral lesions, halitosis, increased calculi, periodontal diseases, hairy tongue, and oropharyngeal and respiratory cancers [8]. Saliva being the first biological fluid exposed to cigarette smoke, which consists of numerous toxins, causes structural and function changes in saliva [5,9].

It is known that smoking tobacco influences general and oral health. All in all, oral and dental problems can substantially influence a person's quality of life by undermining his/her physical performance and social performance [8].

Evidence suggests smoking to be one of the external factors which reduces the SFR; however, research findings are contrasting [10,11]. There are conflicting reports about the correlation between cigarette smoking and mouth dryness, as some studies have indicated that cigarette smokers have lower salivary flow rate than non smokers [4,7-10,12,13] whereas other studies have shown that cigarette smoking has no effect on mouth dryness [3,11]. Even today, it is still unclear whether smoking of tobacco has any effect on the salivary flow rate due to presence of limited studies [4,7] in India targeting tobacco in a smoked form as a factor. There is paucity of studies to investigate the SFR in smokers in India. Therefore, the aim of this study was to assess the salivary flow rate in smokers and non smokers and to study the effect of duration and frequency of the smoking habit on the salivary flow rate.

MATERIALS AND METHODS

This analytical cross-sectional study which was conducted on patients reporting to the Outpatient Department (OPD) of Oral Medicine, Diagnosis and Radiology at Mahatma Gandhi Vidyamandir's, Karmaveer Bhausaheb Hiray Dental College and Hospital, Nashik, Maharashtra, India, from January 2019 to January 2020.

An ethical clearance was obtained from the Institutional Ethical Committee (IEC No. 955). A total of 100 patients satisfying the inclusion and exclusion criteria were included in the study.

Sample size calculation: The sample size (n) is derived by using the "Sample size for Frequency in a Population" formula,

$$\text{Sample size (n)} = \frac{[DEFF*Np\ (1-p)]}{[(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]}$$

Where, N=Population size (for finite population correction factor)=60 [7]

p=Hypothesised % frequency of outcome factor in the population= 50%±5 [7]

d=Confidence limits as % of 100 (absolute±%)=5%

DEFF=Design effect (for cluster surveys)=1

$Z_{1-\alpha/2}$ critical value at confidence level of 95%, α is 0.05 and the critical value was 1.96

Therefore, sample size for the present study was 100 (50 in each group). The sample population was grouped as:

Study group: 50 cigarette smokers aged between 18 to 49 years.

Control group: 50 systemically healthy non smokers aged between 18 to 49 years.

Inclusion criteria: The study group and the control group were age and gender matched to eliminate bias. The study group consisted of individuals with a daily habit of smoking atleast 2 cigarettes for a duration of more than 6 months.

Exclusion criteria: Individuals with systemic diseases causing alterations in SFR like diabetes mellitus, diabetes insipidus, salivary gland dysfunction, end stage renal failure, immunocompromised patients, and patients with autoimmune diseases like rheumatoid arthritis, Sjogren's syndrome, lupus erythematous [14] were excluded from the study. Denture wearers, alcohol consumers, tobacco and areca nut chewers, individuals with history of trauma to head and neck, medications altering salivary flow rate and oral malignancy, pregnant or postmenopausal females, individuals undergoing radiotherapy, chemotherapy and passive smokers were also excluded [9].

A detailed case history was recorded to collect the demographics, medical history, habit history and drug history. Details regarding the duration and frequency of smoking habit of the study group were also recorded. Intraoral examination was performed at baseline to check for any ulceration or with recent temporary restorations or sutures which might stimulate salivation. Such patients were excluded from the study.

Procedure

The selected participants were explained about the study and those participants who voluntarily signed the informed consent were recalled as per their suitable day between 9:00 am to 12:00 pm to avoid diurnal variation. They were asked to refrain from smoking, eating, drinking, or brushing their teeth for atleast 2 hours prior to the collection [9]. Stimulated saliva represents the secretion during physiologic stimulation and is present in the oral cavity for about 2 hours whereas unstimulated saliva represents basal salivary flow rate that is present for up to 14 hours a day and is responsible for maintaining the integrity of oral tissues. Therefore, measuring unstimulated salivary secretion is a precise method to analyse SFR [11].

Collection of unstimulated saliva:

- During sample collection, the participants were seated in a comfortable upright position on the dental chair.
- The participants were asked to swallow once to clear salivary secretions that were already present in the mouth to avoid inaccuracy and not to swallow during the test.
- The participants were asked to spit into a sterile plastic container every 1 minute for 5 minutes [9].
- After collection, the salivary flow rate was measured using a graduated glass tube. The average SFR was obtained by dividing the total SFR collected by 5 and was expressed in mL/minute.
- Time was recorded using a Nivia professional digital stopwatch manufactured by Freewill Sports Pvt Ltd.

The salivary flow rate of smokers and non smokers were measured and compared. A comparison of SFR with duration and frequency of smoking habit was performed for the study group, the data for which was recorded and tabulated.

STATISTICAL ANALYSIS

Using Statistical Package for Social Sciences software version 21.0 for Window (SPSS Inc, Chicago, IL), mean and standard deviation was calculated and comparison of unstimulated SFR was done among smoker and non smoker group using Unpaired t-test. One-way Analysis of Variance (ANOVA) test was used to compare the duration and frequency of smoking habit with SFR among smokers. A p-value of <0.05 was considered statistically significant.

RESULTS

This study was conducted on 100 individuals. Both the study group and the control group were age and gender matched. In the present study, the mean age was slightly lesser in smoker participants (38.6 years) as compared to non smoker participants (39.3 years), but this difference observed was not significant statistically (p-value=0.514). So, age distribution of participants was comparable among the groups [Table/Fig-1].

Age (years)	N	Mean	SD	t	DF	p-value
Smokers	50	38.6	7.559	0.657	49	0.514
Non smokers	50	39.3	8.154			

[Table/Fig-1]: Comparison age in smokers and non smokers using independent samples (Unpaired t-test).
p-value <0.05 was considered as statistically significant

The proportion of gender in the smoker and non smoker group was also comparable (p-value=0.460995) [Table/Fig-2].

Groups	N	Male	Female	p-value
Smokers	50	47 (46) [0.02]	3 (4) [0.25]	0.460995
Non smokers	50	45 (46) [0.02]	5 (4) [0.25]	
N	100	92	8	

[Table/Fig-2]: Chi-Square test to compare gender among the smoker and nonsmoker group.
p-value <0.05 was considered as statistically significant

On comparison of salivary flow rate among the smokers and non smokers, it was observed that mean salivary flow rate was less in the smoker group (0.37±0.17 mL/min) as compared to the non smoker group (0.6±0.14 mL/min) and this difference observed was statistically highly significant (p-value=0.00001). Therefore, salivary flow rate was significantly reduced in smokers as compared to non smokers [Table/Fig-3].

Variables	N	Saliva flow rate (mL/min)				
		Mean	SD	t	DF	p-value
Smokers	50	0.37	0.17	-7.62	49	0.00001
Non smokers	50	0.6	0.14			

[Table/Fig-3]: Comparison of unstimulated salivary flow rate (SFR) in smokers and non smokers using Independent Samples (Unpaired t-test).
p-value <0.05 was considered as statistically significant

The duration of smoking habit was compared with the salivary flow rate [Table/Fig-4]. It was observed that the mean SFR in smokers with a history of habit greater than 10 years was 0.30±0.16 mL/min in comparison to smokers with a history of habit for 1-5 years where the mean SFR was 0.58±0.15 mL/minute. Thus, the unstimulated mean salivary flow rate decreased

with increase in duration of smoking with statistically significant results (p-value=0.000015).

On comparison of frequency of smoking habit with the salivary flow rate, it was found that the mean SFR in smokers who smoked cigarettes more than five times a day was 0.25±0.01 mL/min in comparison to smokers who smoked cigarettes 2-3 times a day where the mean SFR was 0.45±0.10 mL/min. Thus, the mean unstimulated salivary flow rate decreased with increase in frequency of smoking. This difference was found to be statistically significant (p-value=0.012816) [Table/Fig-5].

Duration of Smoking habit	N	Saliva flow rate (mL/min)				
		Mean	SD	F	df	p-value
1 to 5 years	10	0.58	0.15	14.24	49	0.000015**
6 to 10 years	17	0.34	0.07			
>10 years	23	0.30	0.16			
Total	50	0.37	0.17			

[Table/Fig-4]: Comparison of unstimulated salivary flow rate with duration of smoking using One-way ANOVA test.
p<0.001** statistically highly significant

Frequency of Smoking habit	N	Saliva flow rate (mL/min)				
		Mean	SD	F	df	p-value
2-3 times a day	19	0.45	0.23	4.787	49	0.012816
4-5 times a day	25	0.34	0.10			
>5 times a day	6	0.25	0.01			
Total	50	0.37	0.17			

[Table/Fig-5]: Comparison of unstimulated salivary flow rate with frequency of smoking using one way ANOVA test.
p-value <0.05 was considered as statistically significant

DISCUSSION

The aim of this study was to evaluate the SFR in tobacco smokers and systemically healthy individuals. Dawes C, reported that the flow of unstimulated whole saliva showed a very marked circadian rhythm [15]. Humans exhibits diurnal rhythms, in which the volume of salivary secretion increases during the day in the active phase and decreases at night in the resting phase [16]. The presence of this diurnal variation impacts the normal values of SFR and therefore the time of sample collection would have a high effect on the results. Literature suggests that saliva samples should be collected at the beginning of the working day which is a time when unstimulated SFR shows the most rapid rate of change [15]. Therefore, the collection of unstimulated saliva samples was performed in the morning hours to maintain uniformity and avoid diurnal variation. The results obtained in the present study showed presence of slightly higher salivary flow rate than normal range (0.3-0.5 mL/min) in the study subjects similar to the mean SFR obtained by Rad M et al., (0.38±0.13 mL/min) [9] but on comparison of salivary flow rate in both the groups, it was observed that the mean salivary flow rate in smokers was 0.37±0.17 mL/min and 0.6±0.14 mL/min in non smokers with a statistically significant difference (p-value <0.001). Thus, it was observed that the SFR was significantly reduced in smokers than in non smokers.

Similar results were reported by Rad M et al., where they investigated the effects of long-term smoking on salivary flow rate on 200 participants in which the mean±SD level of SFR was found to be 0.38±0.13 mL/min in smokers and 0.56±0.16 mL/min in non smokers [9]. These results are in conformity with the present study.

On the contrary, Khan KJ et al., observed that long-term smoking did not adversely affect salivary reflex and salivation in which the mean unstimulated salivary flow rate of the control group (0.44±0.04 mL/min) and smoker group (0.49±0.05 mL/min) did not show much, and no statistically significant difference was observed when the smokers were compared with controls [3]. [Table/Fig-6] includes comparison of similar Indian and international studies from literature with the present study study [3-5,7-13].

It is presumed that the heat generated by tobacco smoking affects the blood flow of the mouth over a period decreasing the blood supply and in due course reduces the SFR [8]. Immunoglobulins like IgA along with few other defensive agents in the blood are also altered and reduced in levels due to smoking [8,12]. The salivary parenchyma is affected by the toxins present in cigarettes which subsequently leads to impairment of the functioning of the salivary glands [8]. Carbon monoxide, one of the leading noxious gases

Author name and year published	Place of study	No. of subjects	Groups compared	Parameters assessed	Conclusion
Fenoll-Palomares C et al., 2004 [11]	Valencia, Spain	159	52 healthy males 107 healthy females	Unstimulated SFR, pH and buffer capacity of saliva	Salivary flow rate depends on age (r-value=-0.222, p-value=0.005) and gender (p-value <0.001) and correlates with buffer capacity (r-value=0.736, p-value=0.001). Obesity (p-value=0.969), smoking (p-value=0.147), and alcohol use (p-value=0.933) do not influence salivary secretion.
Rad M et al., 2010 [9]	Kerman, Iran	200	100 smokers 100 non tobacco users	Unstimulated SFR, Caries, gingivitis, tooth mobility, calculus, oral lesions	Long-term smoking significantly reduced SFR and increased oral and dental disorders associated with dry mouth, especially cervical caries, gingivitis, tooth mobility, calculus, and halitosis.
Khan GJ et al., 2010 [3]	Peshawar, Pakistan	40	20 male smokers 20 male non smokers	Unstimulated and stimulated SFR	Smoking did not adversely affect salivary reflex and salivary secretion.
Dyasanoor S et al., 2014 [7]	Karnataka, India	120	60 smokers and 60 healthy subjects	Unstimulated SFR	Smoking significantly reduced the unstimulated salivary flow rate and it significantly increased dry mouth symptoms.
Singh M et al., 2015 [4]	Uttar Pradesh, India	70	35 smokers 35 non smokers	Unstimulated SFR and salivary pH	Long-term smoking significantly reduces the SFR and salivary pH.
Pertrusic N et al., 2015 [10]	Zagreb, Croatia	60	30 smokers 30 non smokers	Unstimulated SFR and oral hygiene index (Silness and Löe, 1964)	Long-term smoking compromises the function of the salivary glands which is reflected in the reduced amount and poorer quality of saliva. In addition, poorer oral hygiene has been found in smokers.
Gurudath S et al., 2015 [5]	Karnataka, India	437	377 with habit of tobacco smoking, or chewing, or both and 60 subjects as controls	Unstimulated SFR and salivary pH	A statistically significant reduction of SFR (p-value <0.001) was observed in habit groups. On comparison of salivary pH, a statistically significant reduction was observed only in smokeless tobacco usage group when compared with control group.
Alaee A et al., 2017 [8]	Tehran, Iran	100	50 cigarette smokers (case) 50 non smokers (control)	Non stimulated SFR	Reduced salivary flow rate is more significant in cigarette smokers than in non smokers. Quantitative amount of saliva in the control group was 24.8±2.4 mm and in cigarette smokers it was equal to 15.8±2.1 mm.
A'yun Q et al., 2021 [13]	Yogyakarta, Indonesia	60	30 electric smoker group 30 non smoker group	Salivary pH and saliva volume	There is a saliva status difference between electric smokers and non smokers with acidic pH and lower saliva volume in electric smoker group.

Nigar S et al., 2022 [12]	Karachi, Pakistan	217	Active smokers (N=54) Passive smokers (N=163)	Unstimulated SFR, IgA, and clinical oral dryness.	Smoking potentially leads to xerostomia associated with active caries. The number of cigarettes had a negative impact on saliva production, IgA levels, the oral health status.
Present study, 2022	Maharashtra, India	100	50 smokers 50 non smokers	Unstimulated SFR and effect of duration and frequency of smoking habit on the SFR.	Salivary flow rate is significantly reduced (p-value <0.0001) in smokers than in non smokers. There was reduction in salivary flow rate with increase in the duration (p-value <0.001) and frequency (p-value=0.012) of tobacco smoking habit.

[Table/Fig-6]: Comparison of similar studies in literature with the present study [3-5,7-13].

in cigarettes, is responsible in reducing the SFR which causes breakdown of vitamin A and thus leads to restriction of the blood flow along with a reduction in bicarbonate ions [7,8,17]. Also, the nicotine present in cigarettes cause variations in the autonomic nervous system by increasing plasma levels of epinephrine and norepinephrine which may result in reduced flow rates [6] while Kanwar A et al., and Sankepalli S et al., suggested that the decrease in SFR among study subjects is presumably because of the effect of nicotine on the taste nerve receptors [18,19].

On comparison of duration of smoking with salivary flow rate, we observed that the SFR significantly decreases with increase in duration of smoking (p-value <0.001) similar to a study conducted by Qamar A et al., where they observed a significant, gradual decline in resting salivary flow rate levels (p-value=0.001) with the increase in duration of tobacco usage in smokeless form [20].

Some studies have indicated that cigarette smoking would initially cause an apparent transient increase in SFR due to increased action of salivary glands in individuals who begin smoking, but with continued use it has been noticed that some individuals develop a tolerance to the effect of smoking on saliva, and hence it reduces SFR [9,21].

On comparison of frequency of smoking with salivary flow rate, the present study results showed that the SFR decreases significantly with increase in frequency of smoking (p-value <0.012). These findings show that the duration and frequency of smoking have an inverse effect on the resting salivary flow rate.

Limitation(s)

This was a preliminary study with a small sample size. Further studies with a larger sample size and objective methods of assessment of SFR are recommended.

CONCLUSION(S)

Based on the results of the present study, it is concluded that salivary flow rate was significantly reduced in smokers when compared to non smokers. It was observed that the salivary flow rate was reduced with increase in the duration and frequency of the smoking habit. Reduced salivary flow rate has a significant impact on oral health and may further lead to several oral complications. Therefore, measures should be taken by counseling the smokers to quit the habit and educating them about the ill-effects of xerostomia.

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