ORIGINAL ARTICLE

Assessment of salivary flow rate and salivary pH in subjects with smoking and smokeless form of tobacco habits

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Abstract

Background: Saliva is a complex secretion of the oral cavity and is the first fluid to get exposed to the harmful constituents of tobacco products. Repeated exposure to tobacco results in structural and functional changes of saliva. Thus, estimation of salivary flow rate (SFR) and salivary pH was done in subjects having smoking and smokeless form of tobacco habit.

Methodology: The study was conducted in a workplace setting. Subjects with the habit of smoking, smokeless tobacco, or combined habits of smoking and smokeless tobacco were included in this study. Schirmer tear strips and pH strips were used to assess SFR and pH.

Results: On comparison of SFR between control group and habit groups, a statistically significant reduction of SFR 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. A significant reduction in SFR and borderline reduction in pH was observed in subjects with lesions.

Conclusion: Assessment of SFR using modified Schirmer tear strips is a non-invasive, inexpensive, easy to perform, and well-tolerated test for measuring dry mouth, which readily identifies patients who are asymptomatic for xerostomia and hyposalivation due to tobacco habits.

Introduction

Habitual "psychoactive substance (PS) use" is defined as the repeated use of PS, despite the knowledge of its negative health consequences.[1] Some of the readily available PSs are alcohol, tobacco, and arecanut.[2] Tobacco, either as smoked or smokeless form, is one of the most commonly used PS.[3] Smoked tobacco forms are cigarettes, cigars, and loose tobacco in pipes or handmade beedies.[4] Smokeless form of tobacco is available as unflavored tobacco, traditional betel quid, and flavored forms, such as Khaini, Zarda, Mishri, and Datun, or is available in combination with arecanut such as Gutkha and mawa.[4,5]

Whether usage of smoked or smokeless tobacco forms have systemic as well as locoregional effects.[6] In oral cavity, saliva is the first biological fluid that gets exposed to the toxic chemicals present in tobacco or arecanut.[5,7] Saliva is a complex secretion of the oral cavity, wherein 93% of volume is secreted by the major salivary glands and remaining 7% is secreted by minor glands.[8] Saliva is composed of 99% of water, 1% of organic and inorganic molecules, and various antibacterial substances. Saliva plays a significant role in maintaining homeostasis of the oral cavity.[8,9]

Daily secretion of saliva ranges from 0.75 to 1.5 L/day. Unstimulated whole salivary flow rate (SFR) is about 0.3–0.5 ml/min. The normal pH of saliva ranges from 6.7 to 7.3. The buffering capacity of saliva is an important factor and it usually correlates with flow rate, decrease in the flow rate tends to decrease buffering capacity thereby decreasing the salivary pH (acidic), making the oral mucosa and dental structures more vulnerable to changes,[10] and thus the quantity and quality of saliva are important in maintaining the integrity of oral health.[11]

Reduction in the quantity of saliva is known as hyposalivation or xerostomia. Xerostomia is the perceived feeling of dry mouth which may or may not be associated with salivary gland hypofunction. It is subjective and can be measured by means of questionnaires. On the other hand, hyposalivation is a demonstrable reduction in SFR that can be measured objectively by collecting saliva over a specified period of time. Assessment of

dry mouth involves patient's history, a dry mouth questionnaire, and oral examination. Unstimulated SFR is either measured using draining and spitting method which is time consuming and requires special equipment and trained personnel. Hence, a more user-friendly and patient acceptable techniques are put forward. One such newer method is the use of Schirmer tear test strips.[12,13]

The present study aims to assess the effect of tobacco on SFR using Schirmer test and salivary pH in subjects having smoking and smokeless form of tobacco habit.

Methodology

The study was conducted in a workplace setting. Subjects with an age range of 20–50 years, with the habit of smoking, smokeless tobacco, or combined habits of smoking and smokeless tobacco were included in this study. Subjects with systemic disease, subjects who were on medication for systemic diseases, subjects who consume alcohol, and subjects with a history of radiotherapy, trauma to head and neck, dentures wearer, pregnant or postmenopausal women, such subjects were excluded from the study.

Further, the study group was categorized into four groups such as:

- Group I (smoking form): Subjects with habit of smoking.
- Group II (smokeless form): Subjects with habit of using smokeless tobacco.
- Group III (combined): Subjects with combined habits of smoking and smokeless tobacco.
- Group IV (control): Healthy subjects with no habits as controls.

The study was conducted between 9 and 12 am to avoid diurnal variation.[14] Subjective data were collected by asking a specific questionnaire to assess dry mouth. Salivary dysfunction of the subjects was evaluated based on the answers obtained.

- Does your mouth feel dry at night or on awakening?
- Does your mouth feel dry at other times of the day?
- Do you have difficulty in swallowing food?
- Do you chew gum, min daily to relieve dryness?

A thorough oral examination was done to identify lesions. To assess the SFR, subjects were asked to swallow the saliva in the mouth before the test and also asked to rest their tongue on the hard palate so that the test strip does not touch the tongue during test. The rounded end of Schirmer tear strips was placed in floor of the mouth region for a duration of 3 min, and the readings of SFR were recorded by noting the moistened calibrations on the Schirmer strips. Further, the pH strips were placed in the floor of the mouth, and change in color was matched with the color coding based on the pH strip and noted accordingly.

Statistical analysis

Statistical analysis was performed using SPSS version. Mean SFR and pH between the groups was performed using one-way ANOVA followed by Turkey's HSD *post hoc* analysis. Karl Pearson correlation test was used to assess the relationship between duration of habits, SFR, and pH with each study group. The level of statistical significance (*P* value) was set at $P < 0.05$.

Results

In our study, a total of 628 subjects were screened in a five workplace settings, 437 subjects were included in the study, and among them, 377 had habits and 60 subjects were taken as controls. All subjects in the study were men and with an age range of 20–50 years, and mean age range was 28.1 ± 6.7 years. 37% of 377 subjects answered "yes" for questions regarding feeling dry mouth during swallowing or eating and frequent sipping of water. These questionnaires helped in the subjective assessment of dry mouth.[14] The mean duration and frequency of habits in tobacco smokers were 9.4 ± 2.1 years and 8–10 ± 3 cigarettes/day or 20 ± 4 beedies/day.The mean duration and frequency of smokeless tobacco habit was 7.3 ± 1.2yrs, with frequency of 2 -3 packs/day for 12 ± 2 times/day. The mean duration for combined tobacco habit was 6.1 ± 1.1 years, with frequency of smoking of 5 ± 2 cigarettes or beedies/day, along with smokeless tobacco use of about 1–2 packs for 5 ± 2 times/day.

The mean age of study participants in Groups I–IV was 31.1 ± 8.8, 28.6 ± 7.5, 27.2 ± 5.4, and 27.3 ± 5.5 years, respectively. The mean SFR of Group I was 32.2 ± 2.5, Group II was 31.8 ± 2.9, Group III was 32.6 ± 2.1, and Group IV was 33.9 ± 1.8. The salivary pH in Group I was 7.0 ± 0.1, Group II was 6.9 ± 0.3, Group III was 7.0 ± 0.2, and Group IV was 7.0 ± 0.0 [Table 1]. On comparison of SFR between Group IV (control group)

Table 1: Comparison of mean salivary flow rate and salivary pH between the study groups

Parameter	Groups	*n*	Mean	SD	SE	Minimum	Maximum	F	*P* value
SFR	Smokers	60	32.2	2.5	0.3	25	35	11.214	<0.001*
	Smokeless	115	31.8	2.9	0.3	22	35		
	Combined	202	32.6	2.1	0.1	27	35		
	Control	60	33.9	1.8	0.2	30	35		
Salivary pH	Smokers	60	7.0	0.1	0.0	6	7	4.566	0.004*
	Smokeless	115	6.9	0.3	0.0	5	7		
	Combined	202	7.0	0.2	0.0	6	7		
	Control	60	7.0	0.0	0.0	7	7		

*Statistically significant, SFR: Salivary flow rate

and Groups I–III (habit groups), a statistically significant reduction of SFR was observed in Groups I–III. On comparison of salivary pH, a statistically significant reduction was observed only in Group II when compared with control group [Table 2]. Among the 377 subjects with habits, 208 subjects did not have lesions and 169 subjects had lesions [Table 3]. In habit group, SFR and pH of subjects without lesions were 33.5 ± 2.2 and 7.9 ± 0.2, respectively. Subjects with lesions SFR and pH was 30.9 ± 2.0, and 6.9 ± 0.3. A significant reduction in SFR and borderline reduction in pH was observed in subjects with lesions [Table 4]. Distribution of different oral lesions and their mean SFR and pH in the habit groups are presented in Table 5.

Discussion

The study was conducted in five related workplace settings, which provided a similar sociodemographic, socioeconomic, and behavioral variables. The mean smoking or smokeless habit duration of subjects was 2–3 years. Previous studies have stated that duration of habits more than 5–7 years was considered as longer duration of the habit.[15] Repeated exposure to tobacco and its harmful constituents results in structural and functional changes of saliva.

Cigarette smoke contains about 4000 bioactive compounds and 300 carcinogenic contents. Nicotine in tobacco acts on specific cholinergic receptors in the brain which causes neural activation, thereby increasing the SFR for the shorter duration. Further, long-term usage of tobacco leads to enhanced epinephrine effect or inactivation of taste receptors by nicotine, thereby depressing the salivary reflex or degeneration of salivary gland.[16] In our study, there was a significant reduction in the SFR in habit groups. In accordance to our study, decreased SFR in subjects with smoking was observed in studies conducted by Rad *et al*.[7] An increase in SFR in short-term smoking was observed in studies conducted by Rehan *et al*.[7]

In recent years, smokeless tobacco has gained popularity as an alternative to smoking due to its availability, low cost, and feasibility to use in public places or in workplace settings. Studies have shown that different forms of chewable tobacco have different effects on SFR. In our study, subjects chewed ghutka (tobacco and arecanut), panmasala (flavored arecanut), and khaini (flavored tobacco). The presence of arecanut products

Table 2: Multiple comparison between groups for salivary flow rate and salivary pH

Parameters	Groups	Group I versus controls	Group II versus controls	Group III versus controls
SFR	*P* value	<0.001*	<0.001*	0.002*
Salivary pH	*P* value	0.96	0.01*	0.78

*Statistically significant, SFR: Salivary flow rate

Table 3: Comparison of mean salivary flow rate and salivary pH between subjects with habit with lesions and without lesions in each study group

Group	Parameters	Lesions	*n*	Mean	SD	Mean different	t	*P* value
Smokers	SFR	Absent	36	33.6	1.9	3.6	7.664	<0.001*
		Present	24	30.0	1.6			
	Salivary pH	Absent	36	7.0	0.0	0.0	1.230	0.22
		Present	24	7.0	0.2			
Smokeless	SFR	Absent	52	33.3	2.8	2.7	5.715	<0.001*
		Present	63	30.6	2.4			
	Salivary pH	Absent	52	6.9	0.2	0.0	1.364	0.18
		Present	63	6.9	0.4			
Combined	SFR	Absent	118	33.6	1.9	2.3	8.829	<0.001*
		Present	84	31.3	1.6			
	Salivary pH	Absent	118	7.0	0.1	0.0	1.253	0.21
		Present	84	7.0	0.2			

*Statistically significant, SFR: Salivary flow rate

Table 4: Comparison of mean salivary flow rate and salivary pH between total number of subjects with lesions and without lesions in habit groups

Groups	Parameters	Lesions	*n*	Mean	SD	Mean different	t	*P* value
Smokers, smokeless, and combined	SFR	Absent	208	33.5	2.2	2.6	11.976	<0.001*
		Present	169	30.9	2.0			
	Salivary PH	Absent	207	7.0	0.2	0.1	2.008	0.05#
		Present	169	6.9	0.3			

*Statistically significant, SFR: Salivary flow rate

Table 5: Frequency distribution of different oral lesions in different study groups and their corresponding salivary flow rate and salivary pH

Lesions	Smokers	Smokeless	Combined	Total	SFR	pH
Speckled leukoplakia	11	1	6	18	29.6	6.8
Homogenous leukoplakia	6	25	43	72	30.5	6.9
Erythroplakia	9	1	3	13	29.4	6.9
Tobacco pouch keratosis	0	31	22	53	30.9	6.9
Oral submucous fibrosis	0	5	6	11	29.7	6.8
Total	26	63	82	169		

SFR: Salivary flow rate

with tobacco causes alteration in the autonomic nervous system by increasing plasma level of epinephrine and norepinephrine which results in decreased SFR. Chronic use of smokeless tobacco causes degenerative changes of minor salivary glands located in the site of placement.[7] SFR in Group II subjects showed a significant reduction than other habit groups. In accordance to our study, SFR was reduced in studies done by Kanwaer *et al*.[17] In contrary, few studies done by Siddabasappa *et al*. showed an increase in SFR.[18]

The present study reveals a significant reduction in mean salivary pH in smokeless tobacco group. pH varies according to the SFR. Higher the SFR, higher the buffering capacity, thus higher the pH and *vice versa*.[19] Salivary buffering capacity depends on three buffer systems: Carbonic acid/bicarbonate system, phosphate system, and protein system. These bicarbonates vary with SFR. According to previous studies, a significant reduction in salivary pH depends on the frequency and duration of tobacco habit**.** Our study results were in accordance to the studies done by Knwar *et al*.[17] In contrary, the salivary pH was increased in a study done by Rooban *et al*.[3] No significant reduction in salivary pH was observed in a study done by Dyasanoor and Saddu.[13]

In this study, there was a significant reduction in SFR and a borderline reduction in salivary pH in subjects with lesions in habit group. Alteration in the quantity and quality of saliva, along with chronic irritation, makes the oral mucosa more vulnerable to changes. Nicotine is a biphasic component which is readily absorbed by the mucous membrane; once it is absorbed, it forms arachidonic acid metabolites which cause an increased cell division and vascular endothelial growth factor and cyclooxygenase which cause an increased proliferation of abnormal epithelial cells and delay the apoptosis.[19] Chronic irritation of smokeless form of tobacco on oral mucosa is mainly due to the lime which is used with arecanut. Lime causes dislodgement of bicarbonate, thereby making saliva acidic and causing free radical injury which leads to microstructural changes in oral mucosal membrane.[20]

Alterations in levels of SFR and pH in smoking or smokeless form of tobacco can impair the salivary defense mechanism. Tobacco use is associated with several mucosal changes from innocent to irreversible changes of the oral mucosa. Hence, quantitative and qualitative assessment of saliva in subjects with smoking/smokeless form of tobacco habit aids in early detection of oral mucosal deterioration. It is decisive from our study that smoking or smokeless form of tobacco has a definitive effect on SFR and salivary pH. Smokeless form of tobacco is more harmful, as SFR and pH are altered more.

Assessment of SFR using modified Schirmer tear strips is a non-invasive, inexpensive, easy to perform, and well-tolerated test for measuring dry mouth, which readily identifies patients who are asymptomatic and those who experience profound xerostomia and hyposalivation due to tobacco habits. Hence, SFR and salivary pH measurement can be used for assessing the early pathological changes in the oral mucosa. Furthermore, research with longitudinal study design is needed in assessing SFR and pH alteration in subjects with smoking and smokeless habits. Further studies are recommended for assessment of SFR and pH in larger sample of patients with tobacco associated oral lesions.

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