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Assessing Salivary Flow Rate, Salivary pH and Oral Candidiasis among Tobacco Chewers, Smokers and Healthy Controls- A Cross Sectional Study

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PP and GR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RR managed the analyses of the study. Author SKP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Tobacco chewing and smoking is one of the most common causes of mortality and morbidity in developed and developing countries. It decreases the sensitivity of taste receptors which in turn leads to altered taste receptors response and changes in salivary flow rate (SFR) and pH. It either alone or in combination with systemic local factors, is associated with increased oral candidal colonization.
Objectives: To assess SFR, salivary pH and oral candidiasis among tobacco chewers, smokers and healthy controls in patients visiting V S Dental College & Hospital Bangalore.
Methods: A total of 90 male subjects aged 20-40years were divided equally into tobacco smokers (group A), chewers (group B), and controls (group C). Saliva of each subject was collected and SFR was expressed in mL/min. Salivary pH was determined using pH strips. Smear was taken from subjects with oral candidiasis and send for microbiological examination. ANOVA, and chi square test was used for stastistical analysis.
Results: The mean (±SD) SFR was 0.66 ml/min (±0.16) in group A, 0.59 ml/min (± 0.34) in group

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B and 0.94 ml/min (± 0.42) in group C, on comparison a non significant difference was found (P =0.256). The mean (±SD) salivary pH of saliva was 6.7 (±0.38) in group A, 6.3 (±0.63) in group B and 7.16 (±0.30) in group C, and the difference was statistically significant (P <0.001). There was no significant association between tobacco habits and oral candidiasis (p value = 0.129).
Conclusions: Tobacco use either smoking or chewing form reduces the salivary flow rate and pH, and there was no significant association between oral candidiasis and tobacco habits.

Keywords: Salivary flow rate; tobacco; salivary pH; oral candidiasis.

ABBREVIATIONS

SFR : Salivary Flow Rate
ECF : Extracellular Fluid
CFU : Colony Forming Unit
AN : Areca Nut
CSC : Cigarette Smoke Condensate
CSH : Cell Surface Hydrophobicity
COR : Concentrated Oral Rinses
BQ : Betel Quid
UWSFR : Unstimulated Whole Salivary Flow Rate
FSS : Fagerstrom Scale Score

1. INTRODUCTION

Tobacco comes from a plant that is native to America, around Peru and Ecuador [1]. Tobacco was introduced to Europe from America in the fifteenth century, first being used in medicinal purposes. Later, it came to be burnt in pipes for pleasure on a large scale in all parts of the world including India. Pipe smoking gave way to the use of tobacco as snuff and in time to cigars and cigarettes varying from country to country, until cigarette smoking became the dominant form in most of the developed countries between the two world war [2]. Tobacco is one of the major toxic agents in our civilization. Tobacco chewing and smoking is one of the most common causes of mortality and morbidity in developed and developing countries in present times [3].

Tobacco kills 6 million people each year. More than five million of those deaths are the result of direct tobacco use while more than 600 000 are the result of non-smokers being exposed to second-hand smoke. Nearly 80% of the world's 1 billion smokers live in low- and middle-income countries. Approximately one person dies every six seconds due to tobacco, accounting for one in 10 adult deaths. Unless urgent action is taken, the annual death toll could rise to more than eight million by 2030 [4]. Currently the prevalence of smokeless tobacco user in India among males is 24.3 and among females it is 2.9. In case of smoker it is 32.9 in males and 18.4 among females [5].

There are clinical and epidemiological evidences regarding the adverse effects of tobacco on oral health [6]. Numerous studies have shown that tobacco use would lead to an increased incidence and severity of periodontal diseases and a higher rate of tooth loss. The adverse effects of tobacco are numerous and tobacco use has been associated with gingival, oral mucosa and dental alterations.

There are also several studies concerning the effect of chewing tobacco and smoking on salivary secretion. Altered whole-mouth salivary flow rate (SFR) has an important role in the patho-genesis of oral and dental diseases [7]. Therefore it is the first biological fluid that is exposed to tobacco, which contains numerous toxic compositions responsible for structural and functional changes in saliva which in turn leads to depressed salivary reflex [8]. Generally it is accepted that long term use of tobacco decrease the sensitivity of taste receptors which in turn leads to depressed salivary reflex [9].

Nicotine is the main ingredient of tobacco, which acts on certain cholinergic receptors in the brain and other organs causing neural activation leading to altered salivary secretion. This might lead to altered taste receptors response and hence to changes in salivary flow rates and salivary pH [10].

Published data have shown that there is an inverse association between salivary flow rates (SFRs) and Candida albicans counts in saliva [11]. It has also been shown that high C albicans counts in saliva are associated with clinical signs of candidiasis [12]. Candida species constitute a part of human oral commensal flora in 2 to 71% of healthy subjects [13]. Different environment factor have been shown to increase asymptomatic oral candidal transmission such as wearing of removal dental prostheses [14],

salivary pH [15] and interaction between candida and other commensal microflora [16].

Whether tobacco should be considered as one of the factors is still a matter of debate. Several previous studies have also reported that tobacco either alone or in combination with other systemic local factor, is associated with increased oral candidal colonization or with the development of oral candidiasis [17]. The present study was carried out to assess salivary flow rate, salivary pH and oral candidiasis among tobacco chewers, smokers and healthy controls aged 20-40 year visiting V S Dental College & Hospital, Bangalore.

2. METHODOLOGY

The study was conducted during Jan 2016- May 2016 for the period of 4 months. Ethical clearance was obtained by the institutional review board of VS Dental College & Hospital. An written and verbal informed consent was obtained from all the study subjects for inclusion and for collection of saliva samples.

Inclusion criteria were those smoking 10-15 cigarettes/ bidi per day for more than 2 yrs in smoker group, tobacco chewers for more than 2 yrs in chewer group, those who never used any kind of tobacco were considered in the control group. We excluded denture wearers, history of radiotherapy, systemic or salivary gland disease, alcohol consumption and immunocompromised patient.

Study participants were selected from the Patients reporting to the Outpatient Department of V.S Dental College and Hospital, Bangalore. A Purposive sampling technique was used to select the study subjects. A total of 90 subjects were divided into 30 in each group: Group 1- Smokers group, Group 2- Chewers group, Group 3- Control Group.

Clinical examination and saliva collection was carried out by a single investigator. To ensure uniform interpretation and application of the criterion for clinical assessment and saliva collection, training and calibration of the investigator was done in the Department of Public Health Dentistry. Before start of the study, intra- examiner reliability was assessed using kappa statistics, the value was 0.91.

A questionnaire was administered to collect the demographic details (annexure1), smoking habit and nicotine dependence using nicotine dependence test (Fagerstrom) for both tobacco smokers [18] and chewers [19]. The questionnaire consisted of 15 questions. On dependency of smoking form of tobacco –(6), smokeless form of tobacco – (9) carried some point/score based on the answer. The subjects were asked to answer the questions as per their experience of tobacco consumption. Minimum score was 0 and the maximum score was 10.

For saliva sample collection patients were asked to refrain from smoking and chewing tobacco for atleast 2 hrs before the sample collection. After a careful oral examination, stimulated saliva was collected under resting conditions from each study participants during 10am to 1 pm to avoid diurnal variation. Each subject was requested not to eat, drink or perform oral hygiene or chew or smoke 60 min before and during the entire procedure. They were asked to chew preweighed (1 g) unflavored paraffin wax for 1 minutes and instructed to spit the accumulated saliva periodically (for one minute) into a sterile graduated test tube fitted with a funnel. During saliva collection subjects were istructed not to speak or swallow. After collection, the SFR was measured and expressed in mL/min. The collected saliva was estimated less than 1 hour for salivary pH and flow rate. Only the liquid component (not the foam) of saliva was measured as flow rate.

Salivary pH was measured immediately after measuring SFR using the Dental Salivary pH Indicator strips (pH 6.5‑9.0, Indicator paper, Cochin). Based on the color change of the indicator paper strip, the pH was assessed in comparison with a color chart. Manufacturer's instructions were followed while measuring salivary pH. Then Smear was taken from the subjects with oral candidiasis and send to Department of Oral Pathology Of V S Dental College & Hospital for pathological and microbiological examination.

2.1 Statistical Analysis

The results were analyzed using SPSS, version 19(SPSS Inc. Chicago, IL, USA) in frequencies and percentages described as basic information. Continuous variables were expressed as mean ±standard deviations and underwent an analysis of variance (ANOVA). Categorical variables were expressed in percentage and underwent a chi square test. The level of statistical significance was defined as $P < 0.05$.

3. RESULTS

The mean age in group A, group B, group C was 26.85, 25.55 and 24.55 years respectively. Among study subjects majority of them 41(45.6%) belongs to 20-25 yr, 21(23.3%) to 26-30 yr, 4(4.4%) to 31-35 yr and 24(26.7%) in 36-40 yr of age group. With regard to education: It was found that, 12(13.3%) were uneducated, 24(26.7%) had secondary education and 54(60%) had profession education. 39(43.3%) were skilled workers, 24(26.7%) were professional and 27(30%) were unemployed. Out of 90 study participants, 46(51.1%) had income >15,000, 29(32.2%) had income 16-30,000, 14(15.6%) had 31-45,000 and 1(1.1%) had > 45,000.

7(23%) smokers and 3(10%) chewers started tobacco habit around 10-15 yrs, 20(66.6%) smokers and 11(36.6%) chewers started around 15-20 yrs, 3(10%) smokers and 9(30%) chewers around 20-25 yrs, 3(10%) smokers and 9(30%) chewers around 20-25 yrs whereas 7(23.3%) chewers and none of the smokers around 25-30 yrs.

With response to the question on smoking somebody at home, 14(46.6%) smokers and 17(56.6%) chewers had reported that, it was used in their household.

When the subjects were questioned about the reason to start tobacco use: only 8(26.6%) smokers responded it as due to curiosity, 9(30%) smokers and 21 (70%) chewers started due to peer pressure, 3(10%) smokers started due to loneliness, 10(33.3%) smokers and 9(30%) chewers just felt like using.

It was observed that from the study subjects, 24(80%) chewers spend daily Rs. <50 on tobacco compared to 2(6.6%) in smokers. Only 27(90%) of smokers and 6(20%) of chewers have spend around Rs. 50-100, 1 (3.3%) smokers, none of the chewers spend around Rs. 100-500.None of study subjects spend Rs. >500 on tobacco.

The findings regarding the tobacco dependency are as follows: None of the smokers and chewers belongs to low dependence. 9(30%) smokers and 4(13.33%) chewers have fragestrom dependence score (FDS) 3-4 (medium dependence), 12(40%) smokers and 21(70%) chewers had FDS 5-7(high dependence), 9(30%) smokers and 5(16.66%) chewers had FDS 8-10 (very high dependence). 4(13.33%) smokers and 9(30%) chewers had reported that they had taste alteration while 26(86.66%) smokers and 21(70%) chewers do not.

The mean SFR ±SD among smoker was 0.6 ± 0.16 ml/minute, chewers it was 0.59 ± 0.34 ml/minutes and in controls it was 0.9 ± 0.42 ml/min. This difference was not significant p value = 0.256.

The mean salivary pH ±SD among smoker was 6.7± 0.38, among chewers it was 6.3± 0.63, and among control group it was 7.1± 0.30. This difference was significant at p value < 0.001. In multiple comparison, post hoc analysis it was found that the difference between smokers and chewers (p value=0.001) and between smokers and control (p value < 0.001) was significant.

It was observed that all smokers were free from oral candidiasis compared to chewers where 2 of them had oral candidiasis and the result was not significant (p value = 0.129).

The mean score of FDS± SD among smokers was 6 ±0.78 and chewers 6.03± 0.55. This difference was not significant p value= 0.027.

Table 1. Distribution of study participants according to demographic characters

Characteristic		n	%
Age	a) 20-25 yrs	41	45.6
	b) 26-30 yrs	21	23.3
	c) 31-35 yrs	4	4.4
	d) 36-40 yrs	24	26.7
Education	a) Uneducated	12	13.3
	b) Secondary	24	26.7
	c) Profession	54	60.0
Occupation	a) Unskilled	39	43.3
	b) Profession	24	26.7
	c) Unemployed	27	30.0
Income	a) <15,000 Rs.	46	51.1
	b) 16-30,000Rs.	29	32.2
	c) 31-45,00Rs.	14	15.6
	d) >45,000Rs.	1	1.1
Total		90	100

4. DISCUSSION

Among the study participants, smokers initiated at an early age compare to chewers. With regard to tobacco habit acquired, tobacco chewers were more influenced by someone in household compared to smokers which in accordance with Sreeramareddy CT et al. [20].

Table 2. Mean comparison of salivary flow rate among smokers, chewers and control groups

Study groups	Mean	SD	F value	p value
Smokers	0.6667	0.16884	1.384	0.256
Chewers	0.5927	0.34631		
Control	0.9400	0.42432		

Table 3. Mean comparison of salivary pH among smokers, chewers and control groups

Groups	Mean	SD	F value	Post hoc analysis	P value
Smokers	6.750	0.3884	12.750	Smokers*chewers (0.001)	<0.001
Chewers	6.317	0.6363		Smokers*controls (<0.001)	
Control	7.167	0.3032			

Table 4. Association between tobacco habits and oral candidiasis

Groups	Candidiasis		Fischer exact value	P value
	Yes	No		
Smokers	00	28	4.091	0.129
Chewers	02	30		
Non smokers	0	30		

Table 5. Mean comparision of fagerstrom dependence score among smokers and chewers group

	Groups	Mean	SD	t value	p value
Fagerstrom dependence score	Smokers	6.00	0.788	-.189	.027
	Chewers	6.03	0.556		

The reason could be that, tobacco sachets are more attractive, colorful, sweet flavor and easily hidden from parents. Easily availability at home, parents and siblings, cost effective, lack of ban on smoking in public places, social norm etc.

It was observed that compared to tobacco chewers; smokers spend more money on tobacco. Majority of the chewers responded that, they started this habit because of peer pressure. While smokers just felt like.

According to nicotine dependence test for smokers, we observed that majority of them smoke during the 1 hour after awakening and also theyhate to give up first cigarette in the morning. Through nicotine dependence test for smokeless tobacco, we found that majority of the chewers take their first dip within 30 minutes after awakening in the morning which is in accordance with Petkar et al. [21].

The mean SFR was found to be low in the group A and group B compared to group C, although it was not significant. This decrease in SFR in group A and group B subjects is probably due to the effect of nicotine on the taste sensation. This finding is in accordance with the study by Rooban et al. [22].

Similarly Kanwar et al. [23] observed significant differences in the mean SFR in the smokers, Chewers and in controls. Singh M et al. [24] also observed low salivary flow rate among smokers than controls. In contrast Khan et al. [25], observed that some individuals develop tolerance to the salivary effects of smoking in the long term use, hence no effect on salivary flow rate.

However, studies have shown that long term consumption of tobacco in any form, especially in chewing form, is one of the major risk factors for reducing saliva, which was observed in the present study [22]. These findings are in consistent with the finding of Rad et al. [26].

A number of studies have showed that cigarette smoking would typically cause a noticeable short term increase in SFRs, because it increases the activity of salivary glands in anyone who begins smoking where as in long term use it has been observed that some individuals develop tolerance to the salivary effect of smoking so it

reduces SFR. And also smoking is one of the risk factors for reducing saliva and xerostomia [25].

Moreover, in the present study it was also observed that the mean salivary pH of saliva, was low in group A and group B compared to group C , and this difference was statistically significant. Group B participants had lowest salivary pH. The reason could be probably because of use of lime in smokeless form, which can react with bicarbonate buffering system and there by the loss of bicarbonate, turning saliva more acidic. The alteration in electrolytes and ions alters the pH as they interact with the buffering systems of saliva. These findings are in consistent with the results of the Khan et al. [9], Rooban et al. [22] and Singh M et al. [24].

In contrast Reddy et al. [27], observed no difference in salivary pH between the chewers and non chewers. SFR influences the pH of saliva. An increase in SFR alters salivary pH by increasing bicarbonate secretion. Which inturn increases the salivary pH.

It was also observed that there was no significant association between tobacco habits and oral candidiasis. The reason might be the strict exclusion and inclusion criteria and these are in accordance with the study by Colman et al. [28], Oliver et al. [29] and Darwazeh AM et al. [30]. In contrast Muzurovic et al. [31], Baboni et al. [32] found significantly higher candida carriage among tobacco users compare to non tobacco users.

The mean fragestrom dependence score (FSS) was found to be almost equal among smokers and in chewers. The present study did not reveal any significant difference between the groups. This finding is in accordance with Petkar P et al. [21] and in contrast with Jadhav K et al. [33].

The limitation in this study was firstly the smaller sample size, secondly the saliva collected at the time period from 10 am to 1 pm so there might be some biological variation in salivary flow rate and pH, since it is difficult to collect all saliva sample at the same time. Thirdly the Fragestrom dependence test questionnaire records only physical dependence, and it taps only a narrow aspect of dependence. So there is a need for an tool that report both physical and psychological dependence. The study recommend further studies with larger sample size and to correlate SFR and pH with various oral diseases. At the drawn of the twenty first century we need to aim at achieving tobacco free society. All the health care professional can work together to achieve this goal and prevent major health issues through tobacco. The dentist should highlight the effect of tobacco use on health especially while counseling young patients to motivate them to quit the tobacco use. We must maximize access to cessation services for all tobacco users and promote further research into improving tobacco cessation programmes.

5. CONCLUSION

It was observed that the Salivary Flow Rate and pH were lower among both smokers (group A) and chewers (group B) compare with controls (group C). From this observation it was concluded that the tobacco habits either smoking or chewing reduces the salivary flow rate and salivary pH, and there is no significant association between oral candidiasis and tobacco habits.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the authors.

ETHICS APPROVAL

The study protocol was approved by the Ethical Committee of VS Dental College and Hospital and was granted ethical clearance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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