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Resting Salivary Flow Rate and pH Decreases in Chewable Tobacco Users

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SB and MAM designed the study and the protocol. Author AQ managed the literature searches and wrote the manuscript. Authors AA and AQ conducted the research, experimental processes and initial data handling. Author NZ did the statistical analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Salivary flow rate and pH may be altered by chewable tobacco, the habit which is becoming a threat for oral cancer epidemic. The objective of the study was to find out the relationship between alterations in resting salivary flow rate (RSFR) and pH, which are early signs of oral health deterioration, with different forms of chewing tobacco products.
Methods: A total of 354 healthy male subjects, consuming any form of chewable tobacco, belonging to low socioeconomic areas of Karachi were selected for this cross sectional study. A questionnaire was used to collect demographic data and details of chewing habits (using since, pack/day, duration of exposure etc.). Resting saliva of every subject was collected for 5min and

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RSFR was expressed in ml/min. Salivary pH was determined by using pH strips (pH 0-14). Data was analyzed on SPSS version 20.

Results: Of 354 subjects, 27.4% were gutka, 24.3% niswar, 24.3% paan and 24% multiple users with mean RSFR as 0.40±0.30, 0.65±0.32, 0.64±0.39 and 0.41±0.25 respectively. Mean resting salivary pH was 6.58±0.78 with the lowest pH; 6.16±0.65 in multiple users. RSFR and pH significantly decreased with increase in packs consumed/day, duration of exposure and duration of usage.

Conclusion: A significant negative correlation was found between RSFR and pH with tobacco chewing.

Keywords: Saliva; tobacco chewing; resting salivary flow rate; salivary pH.

1. INTRODUCTION

Homeostasis of the saliva in the oral cavity is maintained by continuous secretions of major and minor salivary glands [1]. Besides keeping the oral mucosa moist through a thin film, the saliva, with the complexity of its appropriate composition is critically important in the maintenance of oral health. Apart from the protection of the oral cavity from different microorganisms, it also plays a significant role in teeth remineralization, digestion, phonation, taste sensation, lubrication of food bolus and balance of pH [2]. Saliva is 99% water and remaining 1% comprises ions (calcium, sodium, potassium, phosphate), organic compounds (such as uric acid, glucose, fatty acids, amino acids) and proteins like mucin, amylase, glycoproteins, histatins and statherins. Immunoglobulins, lysozymes, lactoferrins, defensins and hormones like cortisol, aldosterone, testosterone, progesterone and estradiol are also found [3].

Resting or Unstimulated Salivary Flow Rate (RSFR) refers to the fluid released in the oral cavity in the absence of any exogenous or pharmacological stimuli. RSFR in healthy individuals is found to be 0.3 – 0.5 ml/min. [4] However, stimulated salivary flow rate, which approximates 80–90% of the daily saliva produced, results from gustatory, olfactory, mechanical or pharmacological stimulus. On average, a healthy individual produces 1–3 ml/min or 1–1.5 L of saliva per day. The salivary flow rate is an important parameter as the composition of saliva markedly depends on it [5]

While the unstimulated saliva keeps the oral cavity hydrated, the saliva's capacity to buffer hydrogen ions under resting conditions is also significant. There are a variety of sources of hydrogen ions in the oral cavity, such as organic and inorganic acids in saliva, oral microbiota and food. Saliva buffering capacity depends on three buffer systems; carbonic acid/bicarbonate system, phosphate system and the protein system. Carbonic acid/bicarbonate and the phosphate system, both regulate pH under resting conditions, however, under stimulated states carbonic acid/bicarbonate system contributes to 90% of the buffering capacity. The concentration of bicarbonate varies with flow rates and is found to be higher at high flow rates [3,6]. Normal salivary pH, under resting conditions, is estimated to be around 6.5–7.5 [7] and this is directly related to salivary flow rates i.e. low pH at low flow rates and vice versa [8].

A Large number of physiological factors (age, gender, circadian rhythm, physical activity, hydration status, tobacco and alcohol abuse, etc.) influence both unstimulated and stimulated salivary flow rate and hence pH. Long term use of drugs, such as anticholinergic, antihistaminic, diuretics and antihypertensive agents, pathological conditions like oral, pharyngeal, autoimmune, nutritional, metabolic or neurological abnormalities are also found to be associated with altered salivary gland function [2,9].

Tobacco, smoked or chewable, is one of the most commonly used psychoactive substances [10]. Several studies over the decades have shown the adverse effects of different forms of tobacco. Chewable tobacco has gained popularity over the years as an alternative to smoking with the perception that it has less adverse effects compared to smoked tobacco. However, in recent years, it has been found to be strongly linked with the declining of oral health [11,12]. A wider variety of chewable tobacco products are used around the world. In Pakistan, the famous forms are ghutka, niswar and paan [13,14]. The relationship of the effect of these chewable products on resting salivary flow rate and pH among healthy individuals is not well established.

It is anticipated that habitual use of chewable tobacco products decreases the RSFR and pH in healthy individuals. The current study was designed to assess the alterations in resting salivary flow rate (RSFR) and pH among normal healthy individuals who are habitual users of any form of chewable tobacco.

2. METHODS

A cross sectional study was designed in which 354 tobacco chewers, who fulfilled the inclusion and exclusion criteria, were selected from different low socio-economic localities of Karachi. Widely consumed chewable tobacco products in Pakistan are gutka, paan and niswar, that is why people who consumed any of these products were recruited. *Gutka* is a mixture of sun-dried roasted, finely chopped tobacco, areca nut, slaked lime and catechu mixed with flavors and sweeteners, is either chewed or held in the mouth for long hours. *Paan (betel nut)* contains tobacco, areca nut, catechu (Acacia catechu) and slaked lime, wrapped in betel leaf (Piper Betel) with some flavors. *Niswar* is a mixture of sun-dried, sometimes only partially cured, powdered local tobacco (Nicotiana rustica), ash, oil, flavoring agents (e.g. cardamom, menthol), coloring agents (indigo) and lime. It is rolled into a small ball, usually placed under the tongue.

2.1 Inclusion Criteria

Participants selected for the study were healthy males between the ages of 18 to 50 years, consuming at least one form of chewable tobacco (gutka, pan or niswar) for at least a year.

2.2 Exclusion Criteria

People who were healthy male tobacco chewers but were suffering from any acute or chronic disease of oral mucosa and teeth or salivary glands, complained of any systemic disease, had received therapeutic radiation or medications which can affect saliva production were excluded from the study. Individuals consuming chewable tobacco for less than a year and who smoked or were addicted to any other substance were also excluded from the study.

After obtaining a written consent, the demographic data was recorded in a questionnaire comprising details of chewable product used such as frequency and duration of eating habit etc. The approval from Ethical Review Committee, Ziauddin University was taken prior to sampling.

2.3 Saliva Collection

Unstimulated whole saliva of all subjects was collected by trained volunteers, in the morning between 9 a.m. to 12 a.m., to avoid diurnal variations. All subjects were instructed to refrain from eating, drinking, and chewing of tobacco for a minimum of 1 hour before saliva collection. Subjects were comfortably seated and, after a few minutes of relaxation, were asked to avoid swallowing and spit all the saliva they produced for 5 minutes into a graduated test tube, through a glass funnel. The whole volume collected was then measured and expressed in ml/min [15].

2.4 Salivary pH

Salivary pH was immediately measured using pH indicator strips (pH 0-14, universal indicator, Merck, Germany). The instructions provided with the pH indicator strips were followed. The strip was dipped for 5sec in the collected saliva and the color change was noted and assessed by comparing it with the standard chart given. The corresponding pH was then noted [16,17].

2.5 Statistical Analysis

Data was entered and analyzed using SPSS Version 20. Frequencies and percentages were taken out for categorical variables. Mean and standard deviation were calculated for numerical variables. The association of resting salivary flow rate and pH with duration of tobacco usage was analyzed through Correlation Regression. Difference of means among groups is assessed by independent t- test and ANOVA. *P* value of less than 0.05 was taken as significant.

3. RESULTS

The 354 subjects included in the study belonged to different ethnicities of Karachi. Out of these, 5.6% were Gujratis, 17.8% Kachi memon, 14.4% Punjabis, 34.7% Pashtun, 9% Urdu Speaking, 4.5% Balochi, 7.1% Bengali, 6.8% Sindhi. The mean age group of the subjects was 29.3±9.3 years. 97 (27.4%) subjects consumed gutka, 86 (24.3%) niswar, 86 (24.3%) paan and 85 (24%) were multiple users (those who consumed more than one chewable tobacco product).

Mean RSFR and salivary pH of the subjects was found to be 0.52±0.34 ml/min and 6.58±0.78 respectively. The mean number of packs consumed per day, duration of chewing and duration of usage is shown in Table 1. Table 2 shows significant difference in mean RSFR and pH among different types of tobacco chewers. A significant negative but weak association between salivary flow rates and pH with duration of tobacco chewing, duration of usage and number of tobacco packs consumed per day was observed (Tables 3 and 4). However, a weak negative and insignificant association was seen between RSFR, pH with duration of usage among niswar chewers.

Tables 5 and 6 shows mean RSFR and pH values in different tobacco products according to various contributing factors. The lowest RSFR (0.27+0.11) was seen in subjects who consumed gutka for more than 10 years (a significant decrease in RSFR was seen in subjects who consumed gutka for more than 10 years). RSFR was also significantly decreased in gutka and multiple chewers who consumed more than 20 packs of gutka/day.

Table 1. The Mean±SD values of RSFR, pH and different variables

	Mean±SD
No. of Packs/ day	9.27±9.52
Duration of chewing (min)	17.32±14.32
Duration of usage (years)	10.09±7.29
Resting salivary flow rate (RSFR)	0.52±0.34
pH	6.58±0.78

A Spearman's rank-order correlation was run to determine the relationship between pH and RSRF. A strong, positive correlation was observed, which was statistically significant (r=.617, p=.001) (Table 7). Salivary pH was significantly associated with duration of tobacco usage, with the lowest pH 5.9±0.65 among multiple users using tobacco for more than 10 years (Table 6). The table also shows that salivary pH is also significantly influenced by the increased number of tobacco packs consumed per day and increased duration of exposure.

4. DISCUSSION

Several studies on the estimation of resting salivary flow rate among healthy individuals without any chewing or smoking habit have shown that the normal RSFR lies in the range of 0.3-0.5 ml/min [18), low range 0.1-0.3 ml/min and levels below 0.1 ml/min is considered as hyposalivation [19-21]. In the present study, the mean RSFR of all the subjects (Table 1), lies in the normal healthy non chewers's reference range and the lowest mean RSFR was observed among gutka and multiple users (Table 2). Siddabasappa et al. [22] observed a high level of RSFR (0.61±0.07 ml/min) in gutkha chewers without oral submucous fibrosis, but their sample size was very small (20 subjects). Rooban et al. [23] also found highest mean RSFR of 4.18 ml/10 min (0.418 ml/min) among raw areca nut chewers, whereas our findings were similar to Kanwar et al. [24] who also found a decrease in RSFR in long term smokeless tobacco chewers.

A significant, gradual decline in RSFR levels with the increase in duration of tobacco usage was observed in this study. The lowest RSFR (Table 5) was seen among those who have been consuming gutka for a period of more than 10 years. Rad et al. [25] also found significantly low RSFR values in chronic smokers. Chewing of tobacco causes increased stimulated salivary flow rates because of the parasympathetic effect which is no longer there during periods of non-chewing. Also literature shows that nicotine and areca nut products cause alterations in the autonomic nervous system by increasing plasma levels of epinephrine and norepinephrine which may result in decreasing flow rates in between periods of chewing [26,27]. This could be one reason for decreased RSFRs among prolonged gutka users. Kanwar et al. [24] suggested that the decrease in SFR among study subjects is probably due to the effect of nicotine on the taste nerve apparatus.

The RSFR among niswar and paan eaters (betel quid) was in the range of stimulated salivary flow rates probably because niswar is a product that is only placed in the buccal mucosa and is sucked not chewed. Paan consumed is with tobacco, though the quantity of tobacco and betel nut in paan is less than that used in gutka resulting in more pronounced effect on salivary secretion. Niswar is a preparation of sun-dried, sometimes only partially cured, powdered local tobacco (Nicotianarustica), ash, oil, flavouring agents (e.g. cardamom, menthol), colouring agents (indigo) and lime [28] and none of the niswar consumers used more than 1packet/day, suggesting that there is a less effect of niswar habit on salivary rate even in the long term.

Table 2. Mean values of Resting Salivary Flow Rate (RSFR) and Resting Salivary pH among different types of smokeless tobacco users

	Gutka (n = 97)	Niswar (n = 86)	Paan (n=86)	Multiple users (n=85)	P- value
RSFR (Mean±SD)	0.40±0.30	0.65±0.32	0.64±0.39	0.41±0 .25	0.0001
pH (Mean±SD)	6.60±0.79	6.89±0.55	6.65±0.93	6.16±0.6 5	0.0001

Table 3. Association between Resting Salivary Flow Rate (RSFR) with duration of chewing, number of packs consumed/day and duration of tobacco usage

RSFR	Duration of chewing (min)			Packs/day			Duration of usage (years)		
	r	Un-standardized beta	P-value	r	Un-standardized beta	P-value	r	Un-standardized beta	p-value
Gutka	0.184	-0.007	0.0001	0.102	-0.004	0.0001	0.350	- 0.016	0.001
Niswar	0.234	-0.013	0.0001	0.208	-0.124	0.0001	0.119	- 0.005	0.275
Paan	0.219	-0.006	0.0001	0.225	-0.010	0.0001	0.205	- 0.012	0.058
Multiple users	0.330	-0.008	0.0001	0.356	-0.013	0.0001	0.244	- 0.008	0.024

Table 4. Association between resting salivary pH with duration of chewing, number of packs consumed/day and duration of tobacco usage

pH	Duration of chewing (min)			Packs/day			Duration of usage (years)		
	r	Un-standardized beta	P-value	r	Un-standardized beta	P-Value	r	Un-standardized beta	P-value
Gutka	0.141	-0.014	0.0001	0.359	-0.039	0.0001	0.478	- 0.058	0.001
Niswar	0.228	-0.026	0.0001	0.232	-0.286	0.0001	0.118	- 0.008	0.280
Paan	0.155	-0.011	0.0001	0.206	-0.024	0.0001	0.447	- 0.064	0.001
Multiple users	0.376	-0.021	0.0001	0.488	-0.040	0.0001	0.310	- 0.026	0.004

Table 5. Mean RSFR in different tobacco products according to various contributing factors

		RSFR Mean±SD			
		Gutka n= 97	**Paan n= 86**	**Niswar n= 86**	**Multiple user n= 85**
Using since	<5 years	0.54±0.38	0.73±0.36	0.65±0.32	0.57±0.31
	5-10 years	0.38±0.29	0.65±0.45	0.76±0.41	0.37±0.20
	>10 years	0.27±0.11	0.56±0.37	0.58±0.22	0.35±0.21
	Overall	0.41±0.30	0.65±0.37	0.66±0.32	0.41±0.25
P- value		**0.0001**			
Packs/day	<10 packs	0.46±0.32	0.72±0.40	0.66±0.32	0.46±0.29
	10-20 packs	0.26±0.12	0.52±0.33	-	0.44±0.22
	>20 packs	0.17±0.06	0.31±0.19	-	0.29±0.16
	Overall	0.41±0.31	0.64±0.39	0.66±0.32	0.41±0.25
P- value		**0.0001**			
Duration of exposure	<10 min	0.52±0.37	0.68±0.41	0.72±0.32	0.60±0.47
	10-20 min	0.31±0.14	0.62±0.41	0.45±0.25	0.56±0.31
	>20 min	0.35±0.28	0.46±0.15	0.41±0.17	0.35±0.18
	Overall	0.41±0.31	0.64±0.39	0.67±0.32	0.41±0.25
P- value		**0.0001**			

Table 6. Mean pH in different tobacco products according to various contributing factors

		pH Mean±SD			
		Gutka n= 97	**Paan n= 86**	**Niswar n= 86**	**Multiple user n= 85**
Using since	<5 years	7.19±0.66	7.03±0.89	6.97±0.52	6.52±0.67
	5-10 years	6.34±0.75	6.59±0.79	7.0±0.56	6.20±0.49
	>10 years	6.17±0.57	6.31±0.93	6.68±0.56	5.9±0.65
	Overall	6.6±0.79	6.65±0.93	6.89±0.55	6.16±0.65
P- value		**0.0001**			
Packs/day	<10 packs	6.74±0.78	6.91±0.81	6.89±0.55	6.32±0.66
	10-20 packs	6.26±0.70	6.21±0.92	-	6.24±0.59
	>20 packs	6±0.76	5.33±0.51	-	5.8±0.57
	Overall	6.6±0.79	6.66±0.93	6.89±0.55	6.16±0.65
P- value		**0.0001**			
Duration of exposure	<10 min	6.75±0.80	6.86±0.87	7.0±0.49	6.6±0.54
	10-20 min	6.48±0.72	6.44±0.86	6.5±0.63	6.3±0.78
	>20 min	6.46±0.96	5.87±0.99	6.66±0.57	6.07±0.60
	Overall	6.6±0.79	6.65±0.93	6.89±0.55	6.16±0.65
P- value		**0.001**			

Table 7. Correlation between RSFR and pH

			pH	**RSFR (ml/min)**
Spearman's rho	pH	Correlation coefficient	1.000	.617**
		Sig. (2-tailed)	.	.000
		N	354	354
	RSFR (ml/min)	Correlation coefficient	.617**	1.000
		Sig. (2-tailed)	.000	.
		N	354	354

***. Correlation is significant at the 0.01 level (2-tailed)*

We also observed a significant decrease in RSFR with increase in the number of packs consumed/day and with increased duration of exposure (p=0.0001) among all types of tobacco chewers (Table 5). Low range RSFR is observed in gutka, paan and multiple users who consumed more than 20 packs/day (Table 5). This was similar with observations of Rooban et al. [23], who found low range RSFR (2.56 mL/10 min; 0.256 ml/min) among long term BQ chewers. This was in contrast with Siddabasappa et al. [22] who found increased RSFR among gutka chewers. A study done on long term smokers also showed a significant reduction in RSFR values [25] whereas Khan et al. [29] reported no significant difference in RSFRs of smokers and non-smokers. The estimation of RSFR is significant as the basal saliva and its composition are interpreters of salivary gland status and oral health [30]. A number of studies have shown that smoking reduces saliva secretion [25], chewing tobacco increases stimulated saliva secretion, [31,23] but the effect of chewing on RSFR is still inconclusive as little literature is available. Studies done on the estimation of RSFR among tobacco chewers have limitations of sample size and fail to mention any relation with the duration of tobacco usage or number of packs consumed. [22-24,31] A recent study on tobacco betel-lime quid chewers in Pakistan showed no significant difference in RSFRs of chewers and non-chewers [31]. Khan et al. [32] observed that individuals develop tolerance to the salivary effects of smoking.

Normal salivary pH is from 6 to 7 and varies in accordance with the salivary flow [3]. Higher the flow rate, higher is the buffering capacity and so a higher pH and vice versa [8,18]. Mean pH levels of tobacco chewers in our study lies in this range. We found a strong, positive and significant correlation between RSFR and pH (Table 7). [2,3,24,29] Our results further show a significant decrease in resting salivary pH with the increase in tobacco usage. Lowest pH was observed among multiple chewers consuming tobacco for more than 10 years.

Salivary pH is also significantly reduced with increase in number of packs consumed/day and increased duration of exposure (Table 6). This shows that the pH turns acidic with long term use of smokeless tobacco, thereby decreasing the buffering capacity of oral mucosa. The buffer capacity of saliva relies mainly on levels of bicarbonate ion [16,18,33]. Higher the SFR higher is bicarbonate ion. Rooban et al. [23] and Kanwar et al. [24] has found acidic pH among tobacco users. It is suggested that lime in smokeless tobacco reacts with the buffering system and loss of bicarbonate turns the pH acidic. Khan et al. [29] reported decreased salivary pH among smokers. The results are in contrast with Siddabasappa et al. [22] and Reddy et al. [34] who found no significant difference in pH of chewers and non-chewers.

Studies have shown that RSFR is decreased with prolonged tobacco use in any form (smoked or smokeless) probably by enhanced epinephrine effect or inactivation of taste receptors by nicotine thereby depressing the salivary reflex [32] or degeneration of the salivary glands [35].

The decrease in RSFR and pH observed in our study is independent of age as we have taken subjects between 18-50 years old. Studies have reported that the RSFR remains unaffected in healthy individual till 55-60 years of age [36,37]. Another study reported that reduced RSFR in elderly people usually occurs above 60 years and that too because of increased use of medication or some systemic disorders that occur by that age [38]. Since our participants did not have any dental or oral health issues and had no other risk factors that are known to alter salivary gland function except tobacco use, we can say that the changes in RSFR and pH observed are attributable to long term tobacco chewing. We found a significant negative weak association between RSFR and tobacco use. Also a negative significant association is seen between salivary pH and smokeless tobacco use in the current study.

5. CONCLUSION

A significant negative association was found between RSFR and pH with tobacco chewing suggesting that notable decrease in RSFR and pH occurs with increased tobacco usage in the chewable form. Alterations in these parameters could be an early sign of oral mucosal deterioration. More studies with larger sample size are required to be done to establish a stronger association between these parameters.

6. LIMITATIONS

One limitation of the study was that it lacks age matched, non-tobacco chewers group. We compared our results with the normal RSFR ranges mentioned in the literature. However, had the control group been taken, we could have a

better comparison as the subjects and the controls RSFR and pH values would have been assessed under the similar physiological conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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