Original Article

Habit-associated salivary pH changes in oral submucous fibrosis: A cross-sectional study

ABSTRACT

Background: Salivary pH plays a significant role in the pathogenesis of various oral diseases and conditions. Chewing of areca nut and various tobacco products changes salivary pH.

Aim: The aim of the study was to measure the effect of habitual chewing of areca nut and various tobacco products on salivary pH.

Materials and Methods: The present study included 360 individuals (chewers and nonchewers) of age group between 20 and 30 years who visited the Outpatient Department of Hi-Tech Dental College and Hospital. The patient's salivary pH was measured with the help of a digital pH meter before and after chewing areca nut and various tobacco products.

Results: It was observed that, in all the groups of chewers, pH decreased after chewing except in the gutkha and lime chewing group, where pH increased (pH before chewing was 7.43 ± 0.41 and after chewing was 7.51 ± 0.399), the difference was strongly significant ($P < 0.001$). pH was found to be less in lime and tobacco chewers (6.83 ± 0.33) and more in tobacco, betel nut, and lime chewers (7.50 ± 0.41) in comparison to other groups before chewing; the difference was strongly significant ($P < 0.001$). In the mean ± standard deviation, increase in pH was found among chewers (7.32 ± 0.49) as compared to nonchewers (6.99 ± 0.14), which is the control group, and the data were statically significant ($P < 0.001$).

Conclusion: pH is altered in areca nut and various tobacco chewers, rendering the oral mucosa vulnerable to the toxic effects of areca nut and various tobacco products.

Keywords: Areca nut, salivary pH, tobacco products

INTRODUCTION

Oral fluid is mainly composed of saliva. Other components of saliva include gingival cervical fluids, mucosal transudate, dead cells, bacteria, and food remains.[1] Saliva is secreted from salivary glands. The source of saliva is interstitial fluid through blood capillaries, which enters the salivary glands and gets modified from isotonic to hypotonic fluid.[2] Saliva is essential for protection, lubrication of oral mucosal tissue remineralization of teeth, digestion, taste sensation, stimulation, washed-out effect, pH balance, and phonation.[3] Salivary nucleus in the medulla oblongata is the salivary center which is regulated by the control center in the hypothalamus.[4] As the salivary gland is innervated by autonomic nervous system, it responds to both parasympathetic and sympathetic stimulus but differently. Parasympathetic impulses are more common and mostly isolated, with varying degree of expulsion from the acinar cells causing salivary secretion. It also promotes myoepithelial cells' contraction causing vasodilatation, thereby increasing

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Received: 03 April 2020, **Revised:** 15 May 2020
Accepted: 08 October 2020, **Published:** 16 March 2021

Access this article online	
Website: www.njms.in	Quick Response Code
DOI: 10.4103/njms.NJMS_39_20	

How to cite this article: Sahu RK,Patro S Nayak B, Bardhan D, Panda S, Rajguru JP. Habit-associated salivary pH changes in oral submucous fibrosis: A cross-sectional study. Natl J Maxillofac Surg 2021;12:78-82.

serous salivary secretion. On the other hand, sympathetic stimulus causes the production of thick concentrated saliva by altering the fluid component.[5-7]

It has been estimated that, worldwide, ~600,000,000 people are area nut chewers.[8] It is the fourth most commonly abused social drug, ranking after nicotine, ethanol, and caffeine.[9] The areca fruits are sun dried for several weeks, after which the fibrous shells are removed and the hard, dry nuts, commonly called betel nut or "supari" in India, are ready for use. Such sun dried varieties of BN are very hard and are cut into small pieces to make it easier to masticate.[10] A flavored and sweetened dry mixture of betel nut, catechu, and slaked lime has become increasingly popular either with tobacco (gutkha or khaini) or without tobacco (pan masala). These products are packaged in small, attractive, and inexpensive sachets. BN chewing leads to increased salivary secretion in chewers only by chemical stimulation but not on mechanical. The chewers showed lower levels of potassium, sodium, and salivary amylase. These changes in salivary components were thought to be due to increased salivary flow with its dilutional effect.[11] Areca nut contains many minerals, namely, copper, manganese, zinc, nickel, and lead. Within moments of chewing, gutkha begins to dissolve and turns deep red in color. Copper content of betel nut products is strongly associated with oral submucous fibrosis (OSMF). AN also contains four very important alkaloids, namely, Arecoline, arecaidine, guvacoline, and guvacine. Arecoline has parasympathomimetic activity, which increases salivary flow rate in AN chewers which further increases the pH of saliva.[9,12] The common oral lesions associated with AN chewing include dental attrition, staining, dental caries, periodontal diseases, lichenoid lesions, betel chewers mucosa, oral leukoplakia, OSMF, and oral squamous cell carcinoma.[13]

As saliva is easily available, is reliable, and noninvasively collected, it is widely being used as diagnostic medium in various diseases.[3,14] Very few studies are available on the relationship of areca nut chewing and salivary parameters.[11,14,15] The present study attempts to document the alteration in salivary pH among five selected groups of chewers and compare them with those in healthy controls.

MATERIALS AND METHODS

The present study was conducted on participants who were chewers and nonchewers and attended the Outpatient Department of Oral Medicine and Radiology Department of Hi-Tech Dental College and Hospital, Odhisa. Three hundred healthy adult participants who gave a history of habitual chewing of areca nut and various tobacco products for at least 4 years before the time of study and sixty healthy adults, who were nonchewers, volunteered to undergo the present study. Only male individuals between the ages of 20–30 years of age were selected. Those with the habit of alcohol consumption, history of any other habits, history of trauma to the head and neck, denture wearers, history of radiotherapy, patients with systemic or salivary gland diseases or under any drug therapy, and patients with any lesions in the oral cavity were excluded from the study. Chewers are divided into five groups, sixty individuals in each group: Group I – betel nut, Group II – tobacco, betel nut, and lime, Group III – tobacco and lime, Group IV – gutkha and lime, and Group V – only gutkha. Areca nut and tobacco product pouches were made weighing 2.10 g, each with the help of electronic weight machine, given to habitual chewers [Figure 1]. Written informed consent was obtained from all the individuals before the sampling procedure. The study design was approved by the institutional ethical committee (Reg No. 403/HDCH/2018). Samples were collected between 9:00 am and 12:00 pm to avoid the diurnal variation in the beaker. Each participant was requested not to eat, drink, or chew 60 min before the entire study. Participants were seated in the dental chair and asked to chew on paraffin tablets. The stimulated saliva produced was collected in a graduated container every 1 min for 10 min to both nonchewers and chewers [Figure 1], and the baseline pH was immediately measured. After 5 min instead of paraffin tablets, areca nut and tobacco products were given to habitual chewers, and again the stimulated saliva was produced, and pH was measured by following the previous step. In both cases, pH was measured using digital pH meter [Figure 2].

Statistical analysis was done by applying analysis of variance to find the significance of study parameters between three or more groups of patients, student's *t*-test (two-tailed, independent) to find the significance of study parameters on a continuous scale between two groups (intergroup

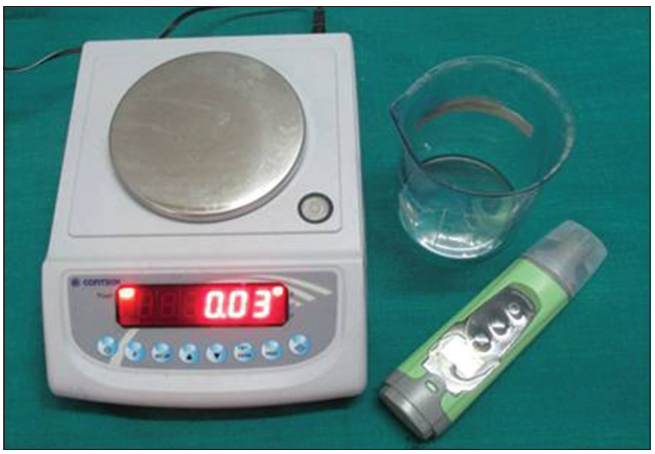


Figure 1: Electronic weight machine, beaker, and digital pH meter

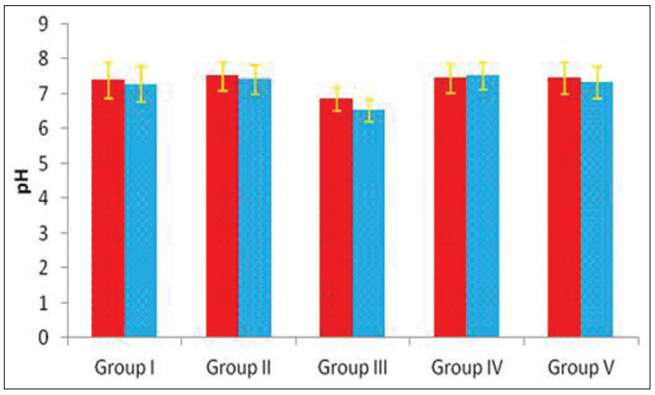
analysis) on metric parameters. *Post hoc* Tukey test was performed to find the pair-wise significance. Chi-square/ Fisher's exact test was done to find the significance of study parameters on categorical scale between two or more groups.

RESULTS

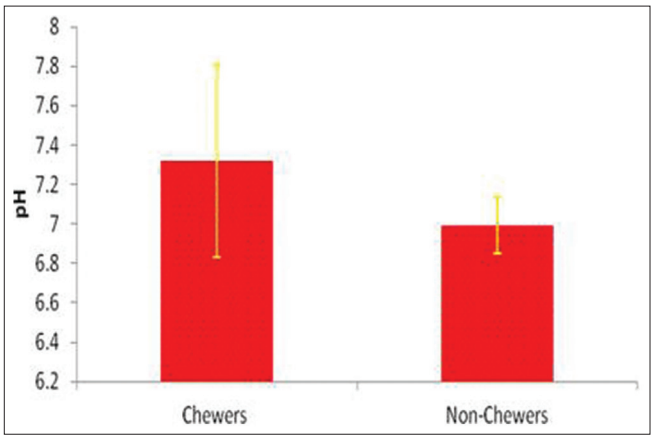
In comparison to all the groups of chewers, salivary pH was found to be increased in Group IV chewers only, i.e., 7.43 ± 0.41 on paraffin-stimulated saliva, and after chewing the areca nut and tobacco product, it was found to be 7.51 ± 0.399, and the difference was strongly significant ($P < 0.001$) [Table 1 and Graph 1]. In Group III chewers, less pH was found (6.83 ± 0.33), and in Group II chewers, more pH was found (7.50 ± 0.41) in comparison with the other groups on paraffin-stimulated saliva, and the difference was strongly significant ($P < 0.001$) [Table 2]. There was a significant ($P < 0.001$) increase in salivary pH among chewers as compared to nonchewers group [Table 3 and Graph 2].

DISCUSSION

In the present study, chewers had an alkaline pH as compared to nonchewers having acidic pH. In chewers, the pH becomes more alkaline after chewing. Rooban *et al*.[14] found that, with chewing raw areca nut, an increase in frequency and exposure time increased pH, respectively. In processed areca nut chewers, an increase in duration and frequency of consumption decreases pH, respectively. For chewers with betel quid with tobacco, increase in duration was significantly associated with a decrease in salivary pH. Kanwar *et al*.[15] in a study divided sixty participants equally into three groups – tobacco smokers A, chewers B, and controls C. The mean pH for Group A – 6.8, B – 6.7, and C – 7.04 when compared and a nonsignificant relation was obtained though, Group A and B showed lower salivary pH. The results show that normal salivary pH was changed to alkaline in chewers because the process of chewing itself brings copious amounts of saliva to the mouth and in the presence of added slaked lime may increase the pH in the oral environment.[9,16] Thus making it alkaline and secondarily due to parasympathomimetic activity of arecoline, areca nut chewers had a high salivary flow rate that also influences the pH of saliva.[12,17] During chewing, it was observed that pH changed from slightly acidic to neutral. These conditions will facilitate the formation of nitrosamines from arecoline, which promotes oral cancer.[18] Alkaline pH is essential for plaque growth that causes periodontal disease.[19] Kang *et al*.[20] found



Graph 1: Comparison of pH in five groups studied (red bar – before chewing, blue bar – after chewing)



Graph 2: Comparison of pH in before and after chewing in all the groups (between chewers vs. nonchewers)

Figure 2: Digital pH meter

Table 1: Comparison of pH in five groups studied

pH	Group I	Group II	Group III	Group IV	Group V	*P*
BC	7.38±0.53	7.50±0.41	6.83±0.33	7.43±0.41	7.44±0.45	<0.001**
AC	7.27±0.52	7.40±0.42	6.51±0.31	7.51±0.39	7.31±0.46	<0.001**

**Significance ($P<0.001$). BC: Before chewing, AC: After chewing

Table 2: Comparison of pH before and after chewing in all the groups (with in group analysis)

pH	BC	AC	Difference	*t*	*P*
Group I	7.38±0.53	7.27±0.52	0.108	5.118	<0.001**
Group II	7.50±0.41	7.40±0.42	0.108	9.653	<0.001**
Group III	6.83±0.33	6.51±0.31	0.323	12.247	<0.001**
Group IV	7.43±0.41	7.51±0.39	0.075	3.866	<0.001**
Group V	7.44±0.45	7.31±0.46	0.127	13.378	<0.001**

**Significance ($P<0.001$). BC: Before chewing, AC: After chewing

Table 3: Comparison of pH before and after chewing in all the groups (between chewers vs. nonchewers)

pH	Chewers	Nonchewers	*P*
Minimum–maximum	6.20-8.30	6.60-7.30	-
Mean±SD	7.32±0.49	6.99±0.14	<0.001**
95% CI	7.25–7.37	6.96–7.01	-

**Significance ($P<0.001$). SD: Standard deviation, CI: Confidence interval

that salivary pH was significantly lower in cancer patients. Lime which is a major component of betel quid preparation causes changes in oral environment of chewers. It changes the pH from neutral to alkaline. Areca nut ingredients release reactive oxygen species (ROS) under alkaline conditions. These ROS are capable of inducing nucleotide modification by forming a compound called 8-hydroxydeoxyguanosine. This compound is responsible for the formation of mutated initiated cells during replication.[10,18,21-24] Among the areca alkaloids such as arecoline, guvacoline, and guvacine, arecoline is the main ingredient responsible for fibroblast proliferation. Under the influence of slaked lime ($Ca[OH]_2$), arecoline get hydrolyzed to arecadine, which has pronounced effects on fibroblasts.[22] Anwar and Saeed[25] found that the pH of saliva in patients who had leukoplakia was acidic and this pH acted as good media for candida growth, while the majority of the salivary pH in normal individuals was alkaline that did not show a significant evidence of candidal growth.

Abdul Khader and Dyasanoor[26] conducted a study to evaluate and compare the salivary flow rate and pH among 135 outpatients (45 areca nut chewers, 45 OSMF, and 45 controls). A statistically significant increase in salivary flow rate (35.7 mm at 3rd min) among areca nut group and a decrease in salivary flow rate among OSMF group (23.4 mm at 3rd min) when compared to apparently healthy participants (30.7 mm at 3rd min) were observed. The mean pH among areca nut, OSMF, and control groups was 6.76, 6.82, and 6.74, respectively, with no statistical significance.

Shubha *et al*.[27] conducted a study to estimate the salivary flow rate and salivary pH in individuals with smoking and smokeless form of tobacco habit. On comparison of salivary flow rate between control group and habit groups, a statistically significant reduction of salivary flow rate was observed in habit groups. On comparison of salivary pH, a statistically significant reduction was observed only in smokeless tobacco user group when compared with control group. A significant reduction in salivary flow rate and borderline reduction in pH was observed in participants with lesions.

CONCLUSION

Alterations in salivary pH were observed in habitual areca nut and tobacco chewers. The alteration was dependent on the type of areca nut and tobacco chewed. The alteration in pH was vital in the causation of various oral diseases.

Acknowledgment

The help of Dr. K. P. Suresh, Scientist (Biostatistics), National Institute of Veterinary Epidemiology and Disease Informatics, Bangalore, and the department of microbiology of our institution is acknowledged.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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