Original Article

Comparison of salivary flow rate and pH between healthy subjects and tobacco and areca nut chewers

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Abstract

Introduction: Tobacco and areca nuts release carcinogens, which cause alterations in saliva. Evaluation of these changes through estimation of salivary flow rate and pH was performed in tobacco and areca nut chewers and apparently healthy subjects.

Material and Methods: The study group for this comparative study comprised 60 subjects with 20 areca nut chewers (group 1), 20 tobacco chewers (group 2), and 20 non-tobacco and areca nut chewers (group 3) in the ages between 18 and 75 years. After collection of saliva from each subject, the salivary flow rate (SFR) was measured by using graduated tubes, whereas salivary pH was measured using a digital salivary pH meter. Tukey HSD *post hoc* test was performed for comparison of mean SFR and mean pH between study group subjects. Analysis of variance (ANOVA) test was used to find the mean difference in SFR and pH in duration, intensity, and frequency among various types of areca nut and tobacco users. A "*P*" value of less than 0.05 was considered as statistically significant.

Results: The mean age among groups 1, 2, and 3 was 37.70 ± 10.44, 39.75 ± 10.16, and 37.90 ± 10.52 years, respectively, with a statistically insignificant difference. The mean salivary flow rate (ml/20 min) was maximum in group 3 (13.23), followed by group 2 (11.75) and group 1 (10.48), with the statistically significant difference as $P < 0.05$. The mean salivary pH was maximum in group 3 (7.07), followed by group 2 (6.86) and group 1 (6.49), with the statistically significant difference as $P < 0.05$.

Conclusion: Long-term use of tobacco and areca nuts in a chewable form can significantly reduce the salivary flow rate and salivary pH. Hence, these measurements can be used as chair side, non-invasive measures for assessing pathological changes in oral mucosa linked to vulnerable effects among people addicted to these adverse habits; thereby, early re-organization can prevent mobility and mortality.

Keywords: Areca nut, salivary flow rate, salivary pH, tobacco

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INTRODUCTION

A psycho-active substance, tobacco, which can make a patient addictive to it, can be used in two ways, smokeless or smoked forms, and also in conjunction with areca nuts.[1] Certain toxic substances, such as reactive oxygen species, free radicals, and reactive nitrogen species, can get released during tobacco smoking, which can lead to transformation of dysplasia into malignancy.[2] Incidence of gingivitis is almost the same in tobacco smokers and smokeless tobacco users when compared to non-users. People having tobacco smoking show a less gingival bleeding as nicotine leads to vasoconstriction. Mutations in tumor suppressor genes such as p53 and Rb can also be induced by carcinogens present in tobacco such as polycyclic aromatic hydrocarbons, nitrosamines, and formaldehyde.[3]

Areca nuts, which constitute four major alkaloids – arecaidine, arecoline, guvacine, and guvacoline[4,5] – can be chewed in the raw form. It can also be consumed as betel quid when the raw form is taken along with betel leaf with added condiments to it.[5] Areca nuts cause a multitude of effects, which comprise cytotoxicity, genotoxicity, and mutagenesis. Suppression of T-lymphocytes occurs as mediated by a decline in glutathione cellular levels, which is further caused by areca nuts. This enhances the oxidation process of oral mucosa, which magnifies the oxidative stress and damage to the genetic material of epithelial cells. Hallucinations as well as delusions can also happen by massive usage of areca nuts. Furthermore, SLUDGE syndrome can also occur, which is a cholinergic condition, expressing as increased bodily secretions such as saliva, lacrimal fluid, and features such as diarrhoea, gastro-intestinal upset, urinary incontinence, and vomiting. Massive use can sometimes look similar to a presentation seen with poisoning by insecticides.[6] Brown to red changes as well as brown crust formation on oral mucosa can also be observed in people who chew areca nuts. Apart from that, areca nut consumption in large amounts can also cause type IV hypersensitivity reactions and lichenoid-type lesions in oral cavity which at times can induce formation of leukoplakia and sub-mucous fibrosis.[6,7] Associated symptoms include reduced opening of mouth by extensive fibrosis, resulting from formation of collagen fibres leading to band development, which histopathologically is observed as hyalinisation in the sub-epithelial area.[6,7]

According to some studies, short-time use of tobacco increases the salivary flow rate (SFR) and increases the concentration of Na^+ as compared to K^+, whereas long-term effects of tobacco usage on SFR and pH of saliva lead to a decrease in the SFR, pH of saliva, and calcium concentration.[8,9] Arecaline has a para-sympathetic mimetic activity which increases SFR, which further increases pH of saliva. Stimulation also affects the quantity of saliva as well as constituents and pH of saliva. Acidic pH promotes the demineralisation of enamel, whereas alkaline pH promotes plaque mineralisation to form calculus. A minor change in the pH value by one limit corresponds to ten-fold change in hydrogen ion concentration of the solution.[10] The demineralisation process (i.e., caries) occurs when the pH of plaque drops below the critical value of 5.5. Whether a lesion develops or not will depend on the balance between two mechanisms, demineralisation and remineralisation. The process of remineralisation is significantly slower than demineralisation in various areas. The pH of saliva plays an important role in the growth and multiplication of oral microflora. The number of acidophilic bacteria increases, whereas the number of acid-sensitive bacteria decreases when the pH of saliva is very low.[11] Ageing leads to decreased SFR because of parenchymal atrophy.[12] In some studies, men showed a greater flow rate than women. There is a correlation between pH changes in the plaque and sugar clearance from the saliva. These changes in pH and the ability of the pH to recover are expressed by Stephan's curve.[13] Thus, the salivary buffering, clearance, and flow rate work in concert to influence intra-oral pH.[14] The present study was performed with an aim to evaluate and compare the SFR and salivary pH among tobacco and areca nut chewers and healthy subjects.

MATERIALS AND METHODS

This comparative study was conducted at the Department of Oral Pathology, Maharaja Ganga Singh Dental College and Research Centre, Sri Ganganagar, Rajasthan, over the period of years from November 2019 to October 2020. The study subjects were included from the patients reporting to the out-patient department of the institute after obtaining institutional ethical clearance from the ethical committee.

Subjects in the age range of 18–75 years with a habit of areca nut chewing and tobacco chewing for more than 5 years and apparently healthy subjects without any of these habits were included in the study. Subjects with any known systemic disorders; patients on drugs such as anti-cholinergics, diuretics, anti-histamines, anti-hypertensives, and anti-psychotics; denture-wearing patients; patients on radiotherapy and chemotherapy; and patients with clinically proven potentially malignant disorders were excluded from the study. All the study participants were explained about the study, and a written informed consent was taken.

The study group was composed of 60 subjects with 20 areca nut chewers (group 1), 20 tobacco chewers (group 2), and 20 non-tobacco and areca nut chewers (group 3). Details of the chewing habits of the subjects were taken and recorded, which included the type of chewing material, frequency per day (more than 5 times or less than 5 times), and duration of chewing (more than 5 years or less than 5 years).

Saliva collection: Salivary collection was performed between 11:00 am and 1:00 pm to avoid diurnal variation. Each subject was requested not to eat, drink, or perform oral hygiene or chew or smoke 60 minutes before and during the entire study. Subjects were then seated in the dental chair and asked to spit on a graduated container for 20 minutes. During saliva collection, subjects were instructed not to speak or swallow. After collection, the SFR was measured and expressed in ml/minute on the graduated tube [Figure 1]. Salivary pH was measured immediately after measuring SFR using a pH meter [Figure 2]. The manufacturer's instructions were followed while measuring salivary pH. The corresponding value in the pH meter was recorded and taken as the salivary pH. The pH meter was standardized after every procedure using a standard protocol, that is, using pH calibration solutions ranging from pH 4 to pH 7 [Figure 2].

After collection of saliva from each subject, SFR was measured by using graduated tubes, whereas salivary pH was measured using a digital salivary pH meter. Tukey HSD *post hoc* test was performed for comparison of mean SFR and mean pH between study group subjects. Analysis of variance (ANOVA) test was used to find the mean difference in SFR and pH in duration, intensity, and frequency among various types of areca nut and tobacco users. A "*P*" value of less than 0.05 was considered as statistically significant.

RESULTS

The present study comprised 60 subjects, out of which 20 were areca nut chewers, 20 were tobacco chewers, and the rest 20 were controls [Figure 3]. All the subjects in groups 1 and 2 were males, whereas in group 3, 70% (14) and 30% (6) of the subjects were males and females, respectively [Figure 4]. The mean age among groups 1, 2, and 3 was 37.70 ± 10.44, 39.75 ± 10.16, and 37.90 ± 10.52 years, respectively, with a statistically insignificant difference [Figure 5].

The mean salivary flow rate (ml/20 min) was maximum in group 3 (13.23), followed by group 2 (11.75) and group 1 (10.48), with a statistically significant difference as

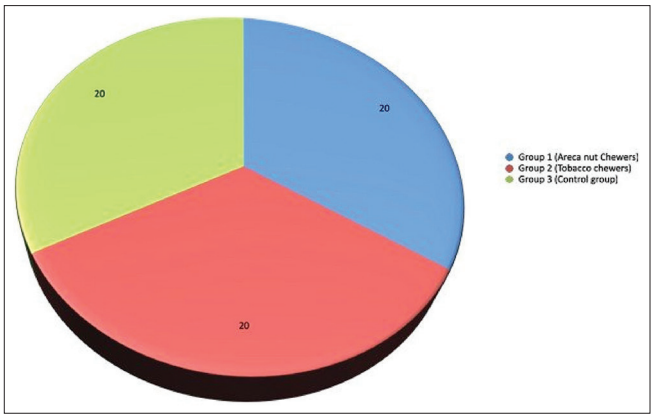

Figure 1: Distribution of patients

Figure 2: Unstimulated saliva sample collection by spitting method

Figure 3: The digital pH meter used for salivary pH analysis and buffering solutions of 4 pH and 7 pH. The tip of pH bulb immersed in the beaker to take the reading

$P < 0.05$. Tukey HSD *post hoc* test revealed that a significant difference was found between all the groups with each other in relation to salivary flow rate (ml/20 min) [Table 1].

The mean salivary pH was maximum in group 3 (7.07), followed by group 2 (6.86) and group 1 (6.49), with a statistically significant difference as $P < 0.05$. Tukey HSD

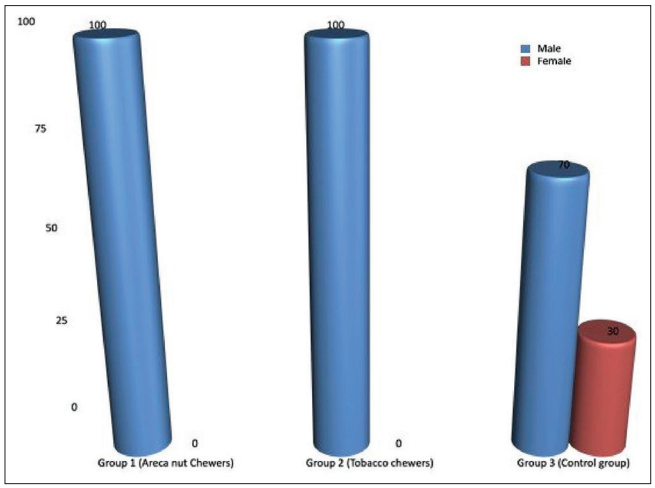

Figure 4: Gender distribution among the study groups

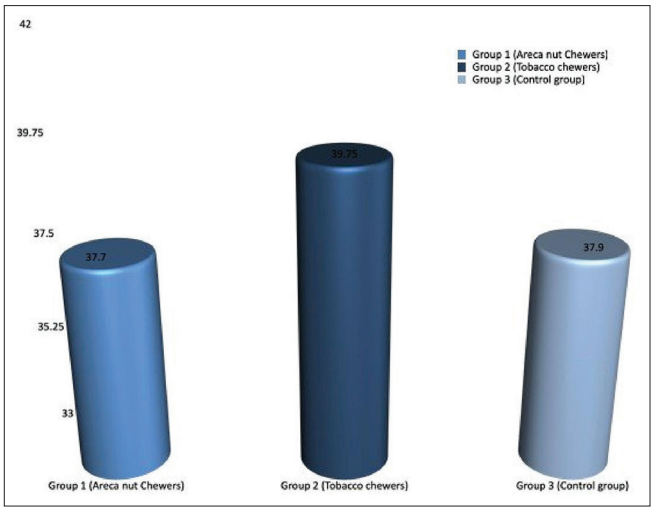

Figure 5: Age distribution among the study groups

post hoc test revealed that a significant difference was found between all the groups with each other in relation to salivary pH [Table 2].

DISCUSSION

A number of studies have shown that tobacco chewing and areca nut chewing would typically cause noticeable changes in the SFR and pH of saliva. In the long term, it is observed that some individuals develop tolerance to the salivary effect (increases the activity of salivary glands), so a reduced SFR is seen in an individual, with long-term use of areca nut and tobacco.

Subjects who chew gutka (tobacco/areca nut), pan masala (flavored areca nut), and khaini (flavored tobacco) show alterations in autonomic nervous system by increased plasma levels of epinephrine and norepinephrine, which result in a decreased SFR and degenerative changes of minor salivary glands located in the site of placement. A significant reduction in mean salivary pH is observed as well. Lime causes dislodgement of bicarbonate, thereby making saliva acidic and causing free radical injury, which leads to micro-structural changes in oral mucous membrane.[1,15] The use of lime is in the smokeless form (areca nut/tobacco), which can react with bicarbonate buffering system by loss of bicarbonate and turns saliva more acidic. The alteration in electrolytes also alters the pH as they interact with the buffering system of saliva. Lime could cause a free radical injury or high alkaline content; it probably reacts with the salivary buffering system and alters the pH. The higher the flow rate, the higher is the buffering capacity and so the higher the pH and vice versa.[16]

An alkaline pH is associated with increased proteolytic activity of Porphyromonas gingivalis. Alkaline pH is favorable for deposition of calcium phosphate, thereby promoting plaque mineralisation. Dental caries is caused by lowered pH (acidic pH), which is favorable for enamel demineralisation.[10]

In the study, it has also been seen that areca nut/tobacco chewers had a poor oral hygiene as compared to the control group. Complaints of gum bleeding, halitosis, and difficulty in mouth opening and swallowing of food were seen in

Table 1: Comparison of flow rate (ml/20 min) among the study groups

Group	Flow rate (ml/20 min)		ANOVA test	*P*
	Mean	SD		
Group 1 (Areca nut chewers)	10.48	1.27	34.65	<0.01*
Group 2 (Tobacco chewers)	11.75	1.11		
Group 3 (Control group)	13.23	0.66		
Tukey HSD *Post-hoc* Test.				
Group 1 vs Group 2: Diff=1.2700, 95%CI=0.4742 to 2.0658, *P*=0.0009*				
Group 1 vs Group 3: Diff=2.7500, 95%CI=1.9542 to 3.5458, *P*=<0.01*				
Group 2 vs Group 3: Diff=1.4800, 95%CI=0.6842 to 2.2758, *P*=0.0001*				

Table 2: Comparison of pH among the study groups

Group	pH		ANOVA test	*P*
	Mean	SD		
Group 1 (Areca nut chewers)	6.49	0.27	40.74	<0.01*
Group 2 (Tobacco chewers)	6.86	0.21		
Group 3 (Control group)	7.07	0.10		
Tukey HSD *Post-hoc* Test.				
Group 1 vs Group 2: Diff=0.3700, 95%CI=0.2134 to 0.5266, *P*=<0.01*				
Group 1 vs Group 3: Diff=0.5800, 95%CI=0.4234 to 0.7366, *P*=<0.01*				
Group 2 vs Group 3: Diff=0.2100, 95%CI=0.0534 to 0.3666, *P*=0.006*				

individuals with the habit of chewing areca nuts, which potentially have a causative role in development of oral lesions and deterioration of oral hygiene and periodontal status.[17] There are studies which describe that initially SFR increases when tobacco is used for a short term. Other studies suggest that SFR reduces with long-term consumption of tobacco in the dry form.[18]

The study of unstimulated salivary secretion is an accurate method to analyse salivary gland status, whereas stimulated saliva is useful for the study of functional reserve.[19] The decreased pH values and increased acidity can be linked with cancer pathogenesis and complication of extensive exposure to radiation therapy/carcinoma of buccal mucosa in particular. In this, uncontrolled growth of tumour cells results in high uptake of glucose by tumour cells, which leads to subsequent anaerobic glycolysis and lactic acid production, thus increasing the acidic environment.[20]

The present study comprised 60 subjects, out of which 20 were areca nut chewers, 20 were tobacco chewers, and the rest 20 were controls [Table 1 and Figure 1]. All the subjects in Group I and Group II are males, whereas in group III, 70% and 30% of the subjects were males and females, respectively [Table I, Figure 1]. The mean age among groups I, II, and III was 37.70 ± 10.44, 39.75 ± 10.16, and 37.90 ± 10.52 years, respectively, with a statistically insignificant difference as the '*P*' value is 0.31 [Table III, Graph III].

In our study, there was a decrease in the SFR among the subjects in group I and group II (11.75 ml/20 minutes) in comparison to the control group (group III) (13.23 ml/20 minutes) [Table IV, Graph IV]. The subjects in group I were the most affected in terms of SFR as their SFR came out to be minimum (10.48 ml/20 minutes) among all the three groups. A statistically significant difference was found between all the groups with each other in relation to SFR [Table IV, Graph IV].

Likewise, a statistically significant difference ($P < 0.05$) was found between all the groups with each other in relation to salivary pH. Group I was not affected (pH = 6.49), followed by group II (pH = 6.86) and group III (pH = 7.07) [Table V, Graph V]. These findings are supported by G. Shubha *et al.* (2018),[1] where they found in their study the results of comparison of SFR between the control group and habit group; group I indicates smoking, group II indicates smokeless tobacco, group III indicates the combined habit of smoking and smokeless tobacco, and group IV is the control group. They found in their study that a significant reduction of SFR was observed in habit in groups on comparison of salivary pH, and a statistically significant reduction was observed in smokeless tobacco usage group, that is, group II, as compared to the control group. Preetika Parmar *et al.* (2017)[4] also highlighted these findings their study, in which subjects were divided into three groups, tobacco smokers (group A), tobacco chewers (group B), and controls (group C). They found in their study that use of tobacco in either the smoking or chewing form reduces the SFR and pH. Alpana Kanwar *et al.* (2013)[21] also analysed and compared long-term effects of tobacco on SFR and pH. Subjects were divided into three groups, tobacco smokers (group A), tobacco chewers (group B), and controls (group C).

The present study indicates that the SFR decreases appreciably among tobacco abusers, especially more among tobacco smokeless form. A lower (acidic) salivary pH was observed in tobacco users as compared with the control. Yashashree Kantak *et al.* (2017)[22] observed the effect of areca nuts and various products of areca nuts on SFR and pH of saliva. Total subjects were divided into areca nut/tobacco chewers (group A) and non-areca nut and tobacco chewers, that is, control group (group B). SFR was found to be altered to a lesser extent in areca nut chewers, and salivary pH was altered to a greater extent in areca nut chewers. T. Rooban *et al.* (2006)[23] performed a cross-sectional study on areca nut chewers and non-chewers, in which subjects were divided into two groups (chewers and non-chewers). The SFR (expressed in ml/10 min) and pH were measured. The difference between the mean SFR for areca nut chewers and non-chewers was not statistically significant, and the mean pH difference was statistically significant. In progressed areca nut chewers, there was a decrease in pH, rendering oral mucosa vulnerable to the toxic effects of areca nuts. Indrani Barman *et al.* (2015)[24] assessed the alteration in SFR and pH parameters among different forms of areca nut chewers (raw/betel leaf), group I, tobacco users (smoking/smokeless form), group II, and control group (group III). Alterations in salivary parameters were observed in different forms of areca nut chewers (group I) and tobacco users (group II). Change depended upon the effect of nicotine, which is linked with the duration of use. The mean SFR of raw areca nut chewers and non-chewers was statistically significant, whereas SFR between areca nut chewers (pan) and non-chewers was statistically non-significant. In reference to pH, the mean pH of (raw) areca nut chewers and non-chewers was statistically non-significant. The mean pH of smoking form/smokeless form (tobacco user group) and non-chewers was also statistically non-significant. Saraswathi Gopal, K *et al.* (2016)[25] analysed and compared the long-term effects of tobacco on SFR and pH among

tobacco chewers/smokers and controls. Patients were categorised into three subgroups: non-tobacco chewers and smokers, tobacco chewers, and tobacco smokers.

From the present study, it was concluded that long-term use of tobacco significantly reduces SFR and salivary pH. These alterations in parameters could be an early sign of oral mucosal deterioration. The decrease in the SFR of tobacco chewers and areca nut chewers in comparison to normal subjects is because of the long term of usage of tobacco and the enhanced epinephrine effect of areca nuts, that is, alteration in the autonomic nervous system by increasing the plasma level of epinephrine and norepinephrine, which results in decreased SFR. Chronic use also causes degenerative changes in minor salivary glands on acinar cells located in the site of placement. Tobacco usage also leads to inactivation of taste receptors by nicotine, thereby depressing salivary reflex.[1,18,21,26] Nicotine also altered secretion of saliva by acting or specific cholinergic receptors in the brain and other organs, causing neural activation.[15,21,22] Reduced SFR is also because of parasympathominetic activity of arecoline, probably because of lime, which converts arecoline to arecaidene.[23] Further analysis showed that the amount of active compounds released during areca nut chewing is absorbed into the circulation and the brain. Possible complex interactions between various absorbed active compounds in the brain and the autonomic nervous system affect the SFR.[23]

The decrease in pH because of areca nut chewing and tobacco chewing is attributed to the presence of lime in smokeless form, which can react with the bicarbonate buffering system, thereby leading to the loss of bicarbonate ions, turning saliva more acidic. The alteration in electrolytes and ions alters the pH[4,5,9] as they interact with the buffering systems of saliva. The salivary pH is negatively correlated with age; that is, as the age increases, the salivary pH decreases.[12] Formation of reactive oxygen species in the oral cavity during betel quid chewing leads to a decrease in pH as well. A decrease in SFR also results in a decrease in pH[26] and vice versa, that is, alteration in electrolyte and ion constituents of saliva,[23] that is, a decrease in bicarbonate content of saliva.[18,19] Lime could cause a free radical injury, or the high alkaline content probably reacts with the salivary buffering system and alters the pH.[25,27] Females usually show a lower SFR and decreased buffer capacity.[12] This difference is explained by the salivary gland size, which is smaller in women. Decreased salivary secretion is also related to a greater frequency of oral dryness seen in females. The pH values of males have been found to be higher than that of females.[12] The alterations in salivary pH can occur in areca nuts and various tobacco chewers and lead to changes in oral mucosa, making it susceptible to toxins released by them.[28]

CONCLUSION

The present study concluded that a long-term use of tobacco and areca nuts significantly reduces the SFR and salivary pH. A notable decrease in SFR and pH occurs with increased tobacco and areca nut usage in chewable form. Alteration in these parameters could be an early sign of oral mucosa deterioration. Hence, SFR and salivary pH measurements can be used as chair side and non-invasive measures for assessing pathological changes in oral mucosa linked to vulnerable effects among people addicted to these adverse habits; thereby, early reorganisation can prevent mobility and mortality.

Consent

Informed written consent was taken from each study participant.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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