Section A-Research paper

Comparing the Role of Areca Nut and Smokeless Tobacco-Related Habit in Changing the Properties of Saliva

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Abstract:

Aims and Objectives: The purpose of this research is to examine how regular use of areca nuts and smokeless tobacco affects salivary qualities such as stimulated salivary flow rate, pH, and buffering capacity. The habitual frequency, length, and exposure to these same factors were also shown to be correlated.

Method: This is a comparative research that used purposeful sampling to recruit 120 people (of either sex) between the ages of 18 and 50. Group A consisted of 60 people who regularly used areca nuts and smokeless tobacco, whereas group B consisted of 60 people who never used these substances. The salivary flow rate, buffering capacity, and pH were measured in both groups to test the hypothesis that these salivary characteristics change as a consequence of the habit and that these changes are related to the frequency, duration, and exposure to the habit. The GC Saliva Check Buffer KitTM was used to analyze saliva samples from these people for salivary flow rate (SFR), pH, and buffering capacity. SPSS version 27.0 was used for the statistical analysis of the data.

Results: The average pH and SFR in group A were 3.25 1.30 units and 6.45 0.15, respectively. There was a statistically significant gap between the two groups, even though group B had a much higher mean stimulated salivary flow rate (4.51) and a slightly higher pH (7.06+0.1).
Conclusion: Habitual chewers of either areca nut alone or areca nut in combination with other products show changes in SFR, as well as in salivary pH and buffering capacity. The nature of the habit, as well as the frequency and length of exposure, determine the degree of change.
Keywords: Areca nut habit, buffering capacity, pH, salivary flow rate, smokeless tobacco habit

Introduction: The mucosa lining the mouth, throat, and even the voice box are constantly bathed in a special bodily fluid called whole saliva. Saliva is a clear, slightly acidic, complex muco-serous secretion that originates in the salivary glands, gingival fold, and oral mucosal transudate. It also contains nasal and pharyngeal mucous, non-adherent oral bacterial, food remnants, desquamated epithelial and blood cells, and traces of medications or chemicals. [1] Hormones, antibodies, growth factors, enzymes, microorganisms, and their products are all present in saliva, just as they are in the serum. Passive diffusion, active transport, and extracellular ultra-filtration allow many of these components to enter saliva from the blood. Saliva is an indicator of how well the body is functioning, as a result. [2]

When the process of secretion of saliva is diminished, the oral tissue becomes susceptible to infection, and the ability to masticate, swallow, speak and taste may be disturbed.[3] Alteration in salivary flow due to any reason can in turn cause changes in its pH, altering the buffering action. Alteration in these parameters has deleterious effects on the oral mucosa and leads to an aggravation of different oral conditions like caries to periodontitis mucosal lesions and also oral cancer. This hurts the quality of life.[4] Areca nut and tobacco products are reported to be substances that can cause mucosal changes. However, their role in the alteration of salivary flow is not very clear. Hence, this study was carried out to evaluate changes if any in the salivary properties of subjects having such deleterious habits and was compared with subjects without any habit.

Materials and Method: The participants in this comparative research were recruited from the outpatient department of a dental hospital after they gave their informed permission in writing to take part. The purpose of the study was to determine whether or not regular use of areca nuts and smokeless tobacco alters the composition of saliva and whether or not these alterations are related to the amount of time spent using these products. The research project received Institutional Ethical Committee approval.

A total number of 120 subjects belonging to the age group of 18-60 were selected using purposive sampling and divided into the following two groups by stratified sampling method: Group A- 60 Subjects having a habit of areca nut with/ without smokeless

tobacco/lime/masala/gutkha for more than one year. Group B- 60 Subjects without any oral adverse habits (control group). They were gender matched with group A. The following subjects were excluded from the study: Subjects with a habit of smoking form of tobacco and alcohol consumption. Subjects had oral lesions which were not associated with the above-mentioned habits. Subjects above 50 years of age, as salivary output, get affected with age. Subjects with an underlying major systemic disease or drug therapy that alter salivary parameters. Subjects who were pregnant. Subjects with a history of radiotherapy.

The participants' mouths were inspected, and their medical histories were recorded, in great detail. Saliva was collected from test participants and analyzed using the GC SALIVA CHECK-BUFFER KIT^{TM} to detect characteristics unique to saliva, such as stimulated salivary flow rate, pH, and buffering capacity. To account for diurnal fluctuation, follow-up with the chosen individuals was place between 9 a.m. and 12 p.m. They were asked to fast for an hour before to the spit test, during which time they were not allowed to consume any food or liquids. The pH of naturally produced saliva was measured, whereas the flow rate and buffering capacity of saliva that had been artificially stimulated were evaluated. After 10 seconds of immersion, the pH strip's color changed to indicate the sample's acidity.

Stimulated whole saliva was collected by chewing on a standardized lump of paraffin. Instructions were given to chew for 1 minute and thereafter to spit out or swallow any saliva produced. The saliva secreted during this 1 minute was not taken for study. The subject was then asked to continue chewing the paraffin block and saliva was collected into the collection cup five times at a regular intervals of 1 minute each. The salivary flow of 5.0 ml at 5 min was considered normal. Using a pipette, sufficient saliva was drawn and one drop was dispensed on the buffering test strip. Results were recorded in 2 minutes.

All the data obtained were recorded in the proforma and subjected to statistical analysis. The collected data were subjected to the Statistical Package for social sciences (SPSS) version 27.0 for statistical analysis.

Results: Table 1 shows the distribution of study subjects (N = 120) according to gender.

Table 1: Distribution of total subjects (*n*=100) in thestudy according to gender

Variables	**Group A (with habit)**	**Group B (without habit)**	**Total**
Total	60	60	120
Males	46	46	92
Females	14	14	28
Mean age	35.1	31.5	33.3

The mean stimulated salivary flow rate among group A was 3.25 ± 1.30 units and pH was 6.45 ± 0.15. Although the mean stimulated salivary flow rate among group B was 4.51 ± 1.42 and the pH was 7.06+ 0.1, the difference in these values was found to be statistically significant.

Table 2 shows that out of 60 subjects, 46 had a habit index of <500, 10 had between 500-1000 and 4 had >1000.

Table 2: Comparison of salivary parameters among different habit index

Salivary parameters		**Habit index**		**Chi-square test, *P***
	<500	500-1000	>1000	
	46	10	4	
Stimulated salivary flow rate				
<3.5 ML	2 (4.3%)	6 (60%)	3 (75%)	<0.001
3.5‑ 5 ML	36 (78.4%)	3 (30%)	1 (25%)	
>5 ML	8 (17.3%)	1 (10%)	0	
PH				
Highly acidic	6 (13.0%)	1 (10%)	1 (25%)	0.002
Moderately acidic	16 (34.7%)	9 (90%)	3 (75%)	
Normal	24 (52.1%)	0	0	
Buffering capacity				
Very low	5 (10.8%)	0	1 (25%)	0.006
Low	9 (19.5%)	8 (80%)	2 (50%)	
Normal	32 (69.5%)	2 (20%)	1 (25%)	

Discussion:

Areca nuts have been linked to an increased risk of mouth cancer. [5,6] However, areca nut's effects are thought to be dose- and habit-dependent, particularly when it comes to saliva. The effects of regular areca nut chewing on saliva's physical qualities have been the subject of less studies and study. [12] On analysis of the demographics of the selected subjects, it was observed

that the habits were more prevalent in males than females as found in other studies.[7] This could be due to sociocultural influences, as well as motives like stress relief, reinforcement, concentration in work, stimulation, and digestion can be attributed to the dependence on this psychoactive substance. [8]

Mawa consists of cured areca nut, with tobacco and slake lime. Consumption of chewing sweetened areca nut was the second most common habit in our study. The flavorful, fragrant, pleasant, affordable, and extremely addicting sweet version of the areca nut. There was also evidence of a regular practice of using packaged areca nut in pan masala and gutkha. These packages consist of areca nut with other substances like lime, spices and flavoring substances. It has been reported that its popularity is gaining among the masses not only because they are convenient and inexpensive but there is aggressive marketing by the companies as well. [9]

It is also known to cause mucosal changes like increased keratinization of the epithelium, or ulcerations as seen in quid induced lesion or oral submucous fibrosis, and even carcinogenesis. In our study, it was found that number of subjects who consumed areca nut with other products was comparatively more. It was also found that the habit in such subjects was more frequent during the day with increased duration and more number of years.[10] Healthy individuals typically produce between 1 and 1.5 L of entire saliva per day.[12] Saliva's secretion and make-up may be affected by a number of external variables. Inducing potentially toxic damage through (1) direct gene mutations, (2) attack salivary proteins and oral mucosa—structural changes—penetration of various objects, and (3) infiltration of inflammatory cells—more ROS—mutation of adjacent cells is possible when chewing on different components of the areca nut. Changes in saliva production might be attributable to these in habitual users. When participants in Group A were asked to enhance their salivary flow, those with habits showed a greater shift toward the lower jaw. S Siddabasappa et al. found similar results in their investigation. [12]

The salivary buffering capacity and pH were found to be low in group A, which is not surprising given that these variables rely on one another. Other investigations have found results consistent with this hypothesis. [12]. In this study, it was found that in Group A those who chewed areca nut alone, had a comparatively higher SFR and pH as compared to chewers of areca nut with other products mainly lime. Increased SFR in areca nut chewers is reported to be due to the parasympathetic activity of arecoline. These findings were also similar to other studies. [13]

Studies have also reported histopathological degenerative changes in the salivary glands structures as there is progression in OSMF. [13] Findings similar to the present study were also observed by Chung-Jung Chiu et al. [14] A negative correlation was found between the salivary properties and frequency duration and exposure of habit. This indicates that there is a moderate

decrease in the salivary flow and the pH. Such similar findings were also found in other studies. [13]

Limitations and Future Prospects The above study indicates deleterious changes in the quality of saliva due to habits of areca and related products. However, the study was conducted in a limited sample confined to a particular geographic area. Further research in a bigger population covering a broader area should be carried out to understand the results better.

Conclusion: Results from this research indicate that people with areca nut and tobacco-related behaviors had a significant decrease in salivary flow, as well as a corresponding alteration in salivary pH and buffering capacity. In addition, a dose-dependent association between oral cavity alterations and areca nut and smokeless tobacco use was found. Saliva alterations were shown to be related to how long an individual had the habit, how often they engaged in it, and how long they were exposed to the substance.

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