

# Assessment of Salivary Flow Rate and pH Among Areca Nut Chewers and Oral Submucous Fibrosis Subjects: A Comparative Study

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**Background:** To assess and compare the salivary flow rate (SFR) and salivary pH among areca nut chewers, oral submucous fibrosis (OSMF) patients and apparently healthy individuals.

**Methods:** A comparative study was conducted to assess and compare the SFR and pH among 135 outpatients (45 areca nut chewers + 45 OSMF + 45 control) at The Oxford Dental College and Research Hospital, Bangalore, India. Subjects were interviewed using structural proforma and Modified Schirmer strips and pH paper were implemented for assessing SFR and pH respectively. Statistical analysis was done using IBM SPSS ver. 21.0 software.

**Results:** A statistically significant increase in SFR (35.7 mm at 3rd minutes) among areca nut group and a decrease in SFR among OSMF group (23.4 mm at 3rd minutes) when compared to apparently healthy subjects (30.7 mm at 3rd minutes). The mean pH among areca nut, OSMF and control groups was 6.76, 6.82, and 6.74 respectively with no statistical significance.

**Conclusions:** The observation and findings of the study clearly showed hypersalivation among areca nut group and hyposalivation among OSMF group, with no significant change in salivary pH when compared to healthy subjects.

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**Key Words:** Oral submucous fibrosis, Saliva

## INTRODUCTION

Oral submucous fibrosis (OSMF) is cause for concern because it is one of the preventable, prevalent, premalignant condition of oral mucosa in India. Based on the clinical and epidemiological studies it has been noted that OSMF is increasingly associated with gutkha chewing habit.<sup>1</sup>

Approximately, 600 million people use areca nut worldwide in some form and is the fourth most commonly used psychoactive substance. Areca nut contains 4 major alkaloids: arecoline, arecaidine, guvacoline, and guvacine. In the presence of lime (calcium oxide, turns to alkali calcium hydroxide in aqueous form), arecoline and guvacoline are largely hydrolysed into arecaidine and guvacine respectively. Arecoline is a parasympathomimetic while arecaidine lacks it. Areca nut can be consumed in the raw

form, or wrapped in betel leaves, lime and other condiments. Tobacco, which is often chewed along with areca nut acts on certain cholinergic receptors in the brain and other organs causing a neural activation.<sup>2</sup>

Several studies of resting salivary pH estimate a range of 5.5 to 7.9, with the higher pH exhibited upon increased salivary flow rate (SFR). The pH of saliva is maintained by the carbonic acid/bicarbonate system, phosphate system and protein system.<sup>2</sup>

Given the paucity of literature on the influence of areca nut chewing on SFR and pH, the present study was intended to assess and compare the alteration in SFR and pH among areca nut chewers, OSMF and healthy subjects.

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## MATERIALS AND METHODS

A comparative study was conducted in outpatient department, the Oxford Dental College and Research Hospital, Bangalore. The study group consisted of 135 outpatients (45 OSMF + 45 areca nut chewers without OSMF + 45 controls) between the age group of 18 to 45 years. The subjects were explained about the procedure involved in the study and informed consent was obtained. The study protocol was approved by the institutional ethical committee.

A detailed case history along with habit of smoking, alcoholism, oral hygiene habits (use of alcohol containing mouthwash) was taken from all the individuals. An informed consent was obtained from all the patients along with the explanation of the procedure to be performed.

A thorough clinical examination was performed and subjects were provisionally diagnosed with OSMF based on clinical features and staged according to Pindborgs criteria.<sup>3</sup> The areca nut chewers and apparently healthy subjects were selected based on the age and gender matching with the OSMF subjects. Control group included subjects without history of habits and systemic illness. The subjects were selected based on the following criteria:

### — Inclusion criteria

- Subjects with a chewing habit of areca nut (raw and commercially available forms of areca nut) Pan masala, Betel quid, Supari, RMD.
- Subjects with clinically proven OSMF (Stage I and Stage II) (burning sensation, restricted mouth opening, palpable fibrous bands).

### — Exclusion criteria

- Individuals above 45 years of age.
- Individuals with other habits like smoking, consumption of alcohol, Ghutka or any other form of tobacco chewing.
- Individuals undergoing treatment for OSMF.
- Individuals with any known stress, systemic illness or pregnancy.
- Individuals under any drugs like anti-cholinergics, diuretics, antihistamines, anti-hypertensives & psychoactive substance that might alter the salivary parameters.

Symptom of xerostomia was recorded using Fox et al.<sup>4</sup> questionnaire. After intraoral clinical examination, the lesion was provisionally clinically diagnosed as OSMF and staged by Pindborgs.<sup>3</sup>

All the participants were asked to refrain from eating, drinking, chewing gum, areca nut chewing and oral hygiene procedures like brushing and mouth rinsing for at least 2 hours prior to the appointment. Assessment of xerostomia, and salivary pH were evaluated between 09.00 a.m. and 11.00 a.m.

The study utilized Schirmer paper (tear touch from Madhu Instruments Pvt. Ltd., New Delhi, India; Medline, Mundelein, IL, USA) (Fig. 1) and pH indicator strips (colormetric paper strips from Carolina Biologicals [pH 5-10], www.carolina.com) (Fig. 2).

### 1. Modified Schirmer test procedure

The subject was asked not to eat or drink 2 hours prior to the modified Schirmer test (MST). After a period of 3 to 5 minutes rest, the patient was asked to swallow all the saliva in the mouth prior to the test, and not to swallow anymore during the test. In addition, the patient was asked to rest the tongue on the hard



Figure 1. Schirmer strip.



Figure 2. pH paper.

palate so that the test strip would not touch the tongue during the test. The MST strip was held vertically with a cotton plier and the round end of the strip was positioned at the floor of mouth. When the round end of the strip contacted moisture, the saliva traveled up the strip and its distance was read at 1, 2, and 3 minutes and recorded immediately (Fig. 3).

– Inference<sup>5</sup>

- Normal SFR: 25-30 mm
- Dry mouth: 10-15 mm
- Mild dryness: 6-10 mm
- Moderate dryness: 2-5 mm
- Severe dryness: 0-1 mm

The test was carried out in the morning hours between 09.00 a.m. to 11.00 a.m. considering the circadian variation.<sup>5,7</sup>

## 2. pH strip method

Salivary pH was measured immediately after measuring SFR using the pH strips. Stimulated saliva was collected from floor of the mouth through the submandibular ducts. The change in color

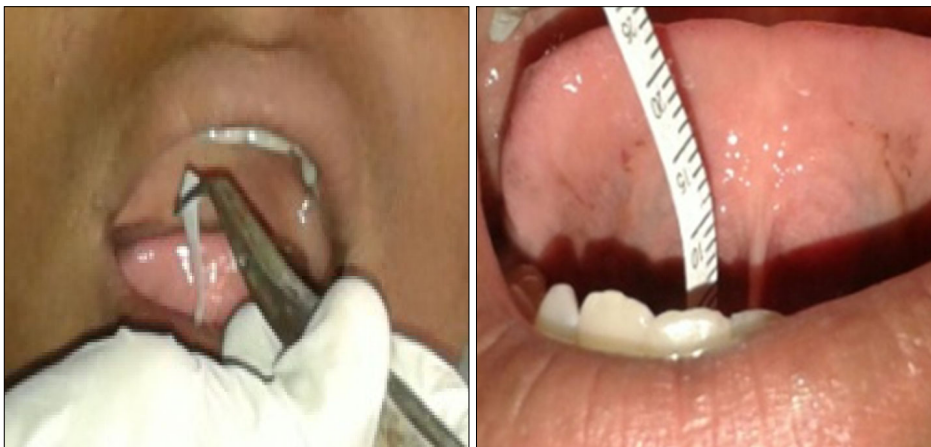
of the strip was noted and matched with the color coding based on the pH strip and noted accordingly (Fig. 4).<sup>5,6</sup>

– Inference<sup>6,7</sup>

- Normal salivary pH ranges from 6.5-7.5.
- If pH value is below 6.5 it indicates an acidic pH.
- If pH value is above 7.5, it indicates an alkaline pH.

## 3. Statistical analysis

Statistical analysis incorporated ANOVA for multiple group comparison and Neumanns Keuls post hoc test for group comparison. Results were expressed as mean  $\pm$  SD and range values. Pearson's correlation coefficient was used to assess the relationship between salivary parameters. The df between variables was also observed. A *P*value of 0.05 was set for statistical analysis. The analysis was carried out using IBM SPSS ver. 21.0 software (IBM Co., Armonk, NY, USA).



**Figure 3.** Salivary flow rate determination.



**Figure 4.** Salivary pH determination.

## RESULTS

Out of 45 OSMF subjects, 39 males (86.7%) with a mean age of 28.7 years and 6 females (13.3%) with a mean age of 29.6 years were observed (Table 1). The areca nut chewers and healthy individuals were matched by the same age and gender as of the OSMF group. The maximum number of OSMF subjects belonging to 21 to 30 years of age group was 25 cases (55.6%), followed by 10 cases (22.2%) in 31 to 40 years of age, 6 cases (13.3%) in 18 to 20 years of age, and the least of 4 cases (8.9%) in  $\geq 41$  years of age. The socioeconomic status (SES) among the three groups revealed 30 cases (66.7%) and 26 cases (57.8%) among the OSMF and areca nut chewers respectively belonging to the lower SES with statistical significance ( $P = 0.00001$ ). Of the control group 27 cases (60.0%) of the individuals belonged to middle SES.

The respondents by frequency, exposure and duration with areca nut chewing habit demonstrated 22 cases (48.9%) of the areca nut chewers and 14 cases (31.1%) amongst OSMF subjects had a habit frequency of maximum 1 to 3 per day. The comparison between the two groups with the exposure of the habit inferred 18 cases (40.0%) among areca nut chewers and 20 cases (44.4%) among OSMF group with a habit usage of  $\geq 11$  minutes. When the respondents were compared with the duration of the habit, 20 cases (44.4%) of the OSMF group used for  $> 5$  years and 17 cases (37.8%) of the areca nut chewers had the habit for a maximum of 1 to 3 years. Statistical significance ( $P = 0.035$ ) was observed with the duration of the habit between the two groups (Table 2).

The comparison of the SFR among the three groups at 1st, 2nd

and 3rd minutes revealed an overall mean of the SFR among areca nut chewers as follows: 17.2 mm, 28.4 mm and 35.7 mm; among OSMF group as 12.8 mm, 19.5 mm and 23.4 mm and among control group as 14.9 mm, 24.2 mm and 30.7 mm, respectively. Pair wise comparison done by Newman-Keuls post hoc test showed statistical significance at 1st minutes ( $P = 0.0116$ ), 2nd minutes ( $P = 0.0042$ ) and 3rd minutes ( $P = 0.0060$ ) among areca nut vs. OSMF subjects suggestive of an increase in SFR among areca nut chewers; statistical significance only at 3rd minutes ( $P = 0.0011$ ) was observed with areca nut chewer vs. control group; statistical significance at 2nd minutes ( $P = 0.0023$ ) and at 3rd minutes ( $P = 0.00001$ ) was observed among OSMF vs. control suggestive of marked reduction of SFR among OSMF group (Table 3, Fig. 5).

Comparison of the 3 groups with respect to salivary pH corroborated the mean  $\pm$  SD of salivary pH among the areca nut chewers to be  $6.76 \pm 0.39$ , OSMF group  $6.82 \pm 0.60$  and control group  $6.74 \pm 0.33$ . Pair wise comparison was performed showing no statistical significance (areca nut group vs. OSMF,  $P = 0.4900$ , areca nut group vs. control,  $P = 0.9084$  and OSMF vs. control group,  $P = 0.6997$ ) (Table 4, Fig. 6).

Correlation of frequency, exposure and duration of SFR among areca nut chewers and OSMF group at the 1st, 2nd and 3rd minutes indicated negative Spearman's coefficient correlation to

**Table 1.** Demographic characters including age, gender and socio-economic status among study samples

Demographic character	Areca nut chewer	OSMF	Control
Age (yr)			
18-20	6 (13.3)	6 (13.3)	6 (13.3)
21-30	25 (55.6)	25 (55.6)	25 (55.6)
31-40	10 (22.2)	10 (22.2)	10 (22.2)
$\geq 41$	4 (8.9)	4 (8.9)	4 (8.9)
Gender			
Male <sup>a</sup>	39 (86.7)	39 (86.7)	39 (86.7)
Female <sup>b</sup>	6 (13.3)	6 (13.3)	6 (13.3)
Socioeconomic status			
Lower class	26 (57.8)	30 (66.7)	9 (20.0)
Middle class	19 (42.2)	14 (31.1)	27 (60.0)
Upper class	0 (0.0)	1 (2.2)	9 (20.0)

Values are presented as number (%).  $P < 0.05$  indicates significant at 5% level of significance (chi-square = 30.3773, df = 4,  $P = 0.00001$ ). OSMF, oral submucous fibrosis. <sup>a</sup>Mean age: 28.7 years. <sup>b</sup>Mean age: 29.6 years.

**Table 2.** Distribution of respondents by frequency, exposure and duration of habit among oral submucous fibrosis (OSMF) and areca nut groups

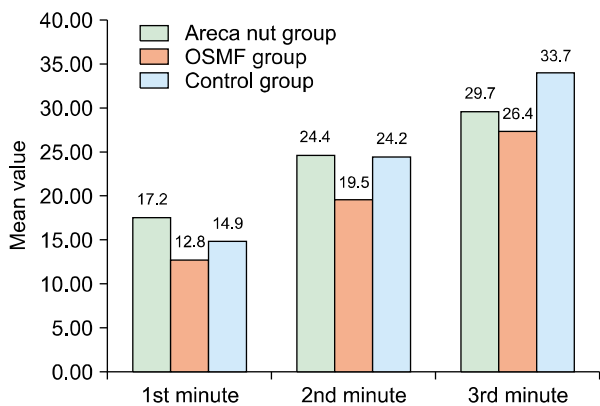
Variable	Areca nut group	OSMF group	Total
Frequency of habit (/day) <sup>a</sup>			
1-3	22 (48.9)	14 (31.1)	36
3-5	14 (31.1)	11 (24.4)	25
5-7	6 (13.3)	10 (22.2)	16
$\geq 8$	3 (6.7)	10 (22.2)	13
Total	45 (100.0)	45 (100.0)	90
Exposure of habit (min) <sup>b</sup>			
$\leq 1$	2 (4.4)	2 (4.4)	4
2-5	13 (28.9)	12 (26.7)	25
5-10	12 (26.7)	11 (24.4)	23
$\geq 11$	18 (40.0)	20 (44.4)	38
Total	45 (100.0)	45 (100.0)	90
Duration of habit (yr) <sup>c</sup>			
$\leq 1$	9 (20.0)	3 (6.7)	12
1-3	17 (37.8)	10 (22.2)	27
3-5	9 (20.0)	12 (26.7)	21
$> 5$	10 (22.2)	20 (44.4)	30
Total	45 (100.0)	45 (100.0)	90

Values are presented as number (%) or number only. <sup>a</sup>chi-square = 6.9072, df = 3,  $P = 0.0749$ . <sup>b</sup>chi-square = 0.1899, df = 3,  $P = 0.9793$ . <sup>c</sup>chi-square = 8.5771, df = 3,  $P = 0.0355$ .

**Table 3.** Comparison of salivary flow rate among the study groups at 1st, 2nd, and 3rd minutes by one way ANOVA

Variable	1st minute (mm)	2nd minute (mm)	3rd minute (mm)
Areca nut group	17.2 ± 9.0	28.4 ± 9.0	35.7 ± 6.6
OSMF group	12.8 ± 8.1	19.5 ± 8.7	23.4 ± 7.1
Control group	14.9 ± 2.7	24.2 ± 2.6	30.7 ± 2.5
F-value	4.1073	6.4876	18.1232
P-value	0.0186*	0.0021*	0.00001*
Pair wise comparisons by Newman-Keuls posthoc test (P-value)			
Areca nut vs. OSMF	0.0116*	0.0042*	0.0060*
Areca nut vs. control	0.1418	0.8978	0.0011*
OSMF vs. control	0.1626	0.0023*	0.00001*

Values are presented as mean ± SD. OSMF, oral submucous fibrosis. \*P < 0.05.



**Figure 5.** Comparison of salivary flow rate among the study groups at 1st, 2nd, and 3rd minutes. OSMF, oral submucous fibrosis.

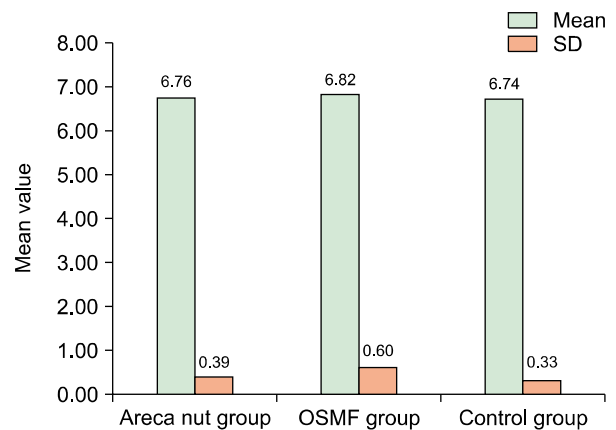
**Table 4.** Comparison of the study groups with salivary pH

Variable	Value
Areca nut group	6.76 ± 0.39
OSMF group	6.82 ± 0.60
Control group	6.74 ± 0.33
F-value	0.3795
P-value	0.6850
Pair wise comparisons by Newman-Keuls posthoc test (P-value)	
Areca nut vs. OSMF	0.4900
Areca nut vs. control	0.9084
OSMF vs. control	0.6997

Values are presented as mean ± SD. OSMF, oral submucous fibrosis.

all 3 habit parameters among areca nut chewers and among OSMF subjects to the habit frequency and exposure, respectively, highlighting a statistical significance (P < 0.05) suggestive of a decrease in SFR with increase in habit parameters (Table 5, 6).

Also, correlation of the habit parameters to salivary pH among



**Figure 6.** Comparison of the study groups with salivary pH. OSMF, oral submucous fibrosis.

**Table 5.** Correlation of habit frequency, duration and exposure on SFR among areca nut groups

Variable	Spearman's R	P-value
Frequency (per day) with SFR at 1st minute	-0.6354	0.00001*
Frequency (per day) with SFR at 2nd minute	-0.6483	0.00001*
Frequency (per day) SFR at 3rd minute	-0.5406	0.0001*
Exposure (in minute) with SFR at 1st minute	-0.5618	0.0001*
Exposure (in minute) with SFR at 2nd minute	-0.5968	0.00001*
Exposure (in minute) with SFR at 3rd minute	-0.5547	0.0001*
Duration (year) with SFR at 1st minute	-0.5465	0.0001*
Duration (year) with salty SFR at 2nd minute	-0.5906	0.00001*
Duration (year) with SFR at 3rd minute	-0.4850	0.0007

SFR, salivary flow rate. \*P < 0.05 is considered statistically significant.

**Table 6.** Correlation of habit frequency, duration and exposure on SFR (measured in millimeter) among oral submucous fibrosis groups

Variable	Spearman's R	P-value
Frequency (per day) with SFR at 1st minute	-0.6128	0.00001*
Frequency (per day) with SFR at 2nd minute	-0.5888	0.00001*
Frequency (per day) SFR at 3rd minute	-0.5794	0.00001*
Exposure (in minute) with SFR at 1st minute	-0.6953	0.00001*
Exposure (in minute) with SFR at 2nd minute	-0.6937	0.00001*
Exposure (in minute) with SFR at 3rd minute	-0.7432	0.00001*
Duration (year) with SFR at 1st minute	-0.2678	0.0754
Duration (year) with salty SFR at 2nd minute	-0.2421	0.1091
Duration (year) with SFR at 3rd minute	-0.1520	0.3188

SFR, salivary flow rate. \*P < 0.05 is considered statistically significant.

areca nut chewers and OSMF subjects revealed negative Spearman's coefficient correlation to habit frequency and duration with statistical significance (P < 0.05) among areca nut chewers

**Table 7.** Correlation of frequency, duration and exposure of habit on salivary pH among areca nut and OSMF groups

Variable	Spearman's R	Pvalue
Frequency (per day) with pH <sup>a</sup>	-0.4699	0.0011*
Exposure (in minute) with pH <sup>a</sup>	-0.6349	0.0000*
Duration (year) with pH <sup>a</sup>	-0.3660	0.0134*
Frequency (per day) with pH <sup>b</sup>	-0.4699	0.0011*
Exposure (in minute) with pH <sup>b</sup>	-0.6349	0.0000*
Duration (year) with pH <sup>b</sup>	-0.3660	0.0134*

OSMF, oral submucous fibrosis. <sup>a</sup>Correlation of frequency, duration and exposure of habit on salivary pH among areca nut groups. <sup>b</sup>Correlation of frequency, duration and exposure of habit on salivary pH among OSMF groups. \* $P < 0.05$  is considered statistically significant.

suggesting a slight decrease in salivary pH among areca nut chewers and to all 3 habit parameters among OSMF subjects (Table 7).

## DISCUSSION

Over the past several decades, dental researchers reported different aspects of OSMF. Yet, there is a big lacunae in the present scenario of evidence based dentistry which correlates the role of critical parameters like SFR and salivary pH among OSMF affected individuals and areca nut chewers.

OSMF is a potentially malignant disease predominantly seen in people of Asian descent. Oral OSMF is a cause for concern because it is one of the preventable, prevalent, premalignant conditions of oral mucosa in India. Based on the clinical & epidemiological studies it has been noted that OSMF is increasingly associated with areca nut chewing habit.<sup>1,8,9</sup>

Most of the dental physicians tend to overlook the changes in SFR and taste changes by focusing more towards the clinical presentation of OSMF and the management of the condition.

During areca nut chewing lot of chemicals & metals like copper, iron are leached out into saliva, which in turn alter the property and composition of saliva. In betel quid chewer's variations in the SFR, pH has been reported. Production of reactive oxygen species is enhanced by the presence of alkaline pH of saliva.<sup>10</sup>

Due to paltriness in literature recording the changes in SFR and pH among areca nut chewers and OSMF subjects with an increase in habit and progression of disease, the present study was therefore aimed in evaluating and comparing the SFR and salivary pH. Previous studies have utilized saliva spitting method whereas, modified schirmer strip method (normal range = 25-30 mm) and pH paper (normal = 6-7.5) were incorporated in the

present study because of its ease in use and a newer implementation for evaluation of taste.

The study group was divided into areca nut chewers, OSMF and control groups who were matched by gender and age as of the OSMF group. Out of 45 OSMF subjects, 14 cases (31.1%) were among stage I and 31 cases (68.9%) stage II.

Our study result showed male: female ratio of 86.7% and 13.3% respectively. Compared to our study varying results were noted in the study conducted by Patil and Maheshwari<sup>11</sup> which showed 53.3% males and 46.7% females being affected by OSMF. The gender distribution in most of the studies varies, ranging from a strong male predominance in different parts of India. They explained that the reason for males to be dominating was as they were using gutkha and other related products more often because of easy availability in all the places whereas females were more conscious about their health and esthetic value, probably feeling uncomfortable to ask the vendors in getting the gutkha products. This is one of the reasons, which may be responsible for a high male to female ratio.<sup>12,13</sup> The contradictory findings in our study may have originated from not considering subjects with ghutka chewing.

In our study maximum number of subjects were seen in the third decade of life followed by fourth decade of life, whereas in a study by Wahab et al.<sup>14</sup> the maximum percentage of subjects (73%) were seen in the second decade followed by third decade of life.

The present study demonstrated the prevalence of areca nut and OSMF among the lower SES which was similar to the studies like those conducted by Ahmed et al.<sup>15</sup> Lower SES was based on their educational attainment, occupational and social classification and income of the subject, which in turn gives them a poor life style and attainment of these habits.<sup>16</sup>

This study revealed duration of more than > 5 year use of areca nut causing OSMF with statistical significance. However, it did not show a statistical significance to habit frequency and exposure in causing OSMF, compared to a study conducted by Reddy et al.<sup>17</sup> which showed the increase in the habit duration, frequency and exposure has caused OSMF ( $P < 0.00$ ).

The present study showed an overall mean of the SFR among areca nut chewers at the 1st, 2nd, and 3rd minutes as: 17.2 mm, 24.4 mm, and 29.7 mm; among OSMF group as 12.8 mm, 19.5 mm, and 26.4 mm; among control group as 14.9 mm, 24.2 mm, and 33.7 mm, respectively. Our study showed a statistically significant increase ( $P < 0.05$ ) in salivary flow among areca nut chewers which could be due to the parasympathomimetic activity of arecoline and arecadine present in areca nut.<sup>18</sup> However, with

increase in the habit parameters (frequency, duration and exposure), decrease in SFR among areca nut and OSMF groups was observed similar to report by Rooban et al.<sup>18</sup>

A decrease in SFR among OSMF subjects could be due to conversion by lime from arecoline to arecadine or due to an atrophy of the acinar cells as disease progresses. Contradictory findings of an increase in SFR among ghutka chewers and OSMF were noted by Siddabasappa et al.<sup>19</sup> using spitting method. The variation of results could be due to utilization of modified schirmer test and use of areca nut habit without tobacco.

The mean pH in the present study among areca nut, OSMF and control groups was 6.76, 6.82, and 6.74 respectively with no statistical significance which was similar to studies conducted by Rooban et al.<sup>18</sup> and Siddabasappa et al.<sup>19</sup> A mild increase in pH among the OSMF group may be due to an increase in the bicarbonate secretion.<sup>18</sup> However, when the habit parameters were correlated with the salivary pH, mild decrease in pH was observed with habit frequency and duration among areca nut chewers. No statistical significance was observed when the OSMF group habit parameters were correlated with pH.

Evaluating methods for SFR and pH using strips are simple chair side investigations and less time consuming. The present study also correlated the habit parameters with salivary parameter among OSMF and areca nut group.

However there are few drawbacks in the present study, which include patient apprehensiveness varying the results to an extent and the selection of OSMF subjects being based on clinical criteria without histopathological confirmation. Also, Stage III OSMF subjects were not included due to insufficient number for the purpose of this study.

OSMF has always been a challenging disease with a high prevalence in India. Areca nut chewing leads to leaching out of chemicals thereby altering the SFR and pH. To conclude it is clear that there are hypersalivation among areca nut chewers and hyposalivation among OSMF subjects. However, with increase in habit duration and exposure there is marked decrease in SFR even among areca nut chewers. More studies have to be conducted on salivary changes among OSMF subjects, as an alteration in these parameters affects the overall quality of life of an individual as well as OSMF having been reported to have malignant transformation ranging from 7% to 13%.<sup>20</sup> Management of these changes can be combined with physiotherapy and pharmacotherapy by the oral physician which can bring satisfaction with an improved quality of life to the patient.

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## CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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