INTRODUCTION
Saliva is necessary for protection, lubrication of oral mucosal tissues, remineralisation of teeth, digestion, taste sensation, stimulation, washed out effect, pH balance and phonation. Saliva is being used for the diagnosis of a wide range of diseases as saliva is proven to be an easily available, reliable and noninvasive diagnostic medium. Salivary parameters are supposed to be altered by drugs like anticholinergics, diuretics, antihistaminics, anti-hypertensive agents and psychoactive substances and conditions like post-surgery, metabolic, nutritional, neurological abnormalities and hydration status.

Resting whole saliva is the mixture of secretions, which enter the mouth in the absence of exogenous stimuli. Several studies of resting whole saliva flow rates (SFR) in healthy individuals have found the value for whole saliva to be about 0.3 to 0.5 mL/minute. The Stimulated salivary flow rate may be as high as 10 mL/minute. Alteration in SFR has a significant impact on otorhodontal health. Altered salivary gland function could be associated with oral, pharyngeal esophageal, neoplastic, metabolic nutritional, inflammatory, genetic, auto-immune and nervous system disorders and require early diagnosis and intervention. It is well known that SFR may greatly vary in an individual and if repeated samples are taken at different time points, varying results will be obtained. Variation in SFR can be as high as 50% over a 24-hour period due to circadian rhythms. Further normal variations have been shown to be age and gender independent.

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

Approximately 600 million people use arecanut (AN) worldwide in some form and is the fourth most commonly used psychoactive substance. The common oral lesions associated with AN chewing include dental attrition, staining, dental caries, periodontal diseases, lichenoid lesions, betel chewer’s mucosa (reddish crusted oral mucosa with
Materials and methods

The duration of study was a month (during August 2003). All the individuals who satisfied the inclusion criteria mentioned below were included in the study. SFR and salivary pH measurements are routinely done chair side, noninvasive procedures that need no laboratory requirements. The whole mouth unstimulated saliva is collected, measured and pH estimated extra orally as described below. Informed verbal consent was obtained from each subject before saliva collection. Subject selection, case history and oral examination were done by one of the authors while other was involved in collection, graduation and measurement of SFR and pH.

Subjects

Subjects included in the study were the individuals attending the outpatient department of our institution. Apparent healthy patients who reported only for oral prophylaxis were included in the study. Pregnant women and patients who had systemic illness, on any drug therapy (within past fortnight) or those who underwent radiotherapy earlier were excluded from the study. 160 healthy males and females were divided into 2 groups as chewers (n=110, using arecanut daily for not less than 6 months) and non-chewers (n=50). Occasional (not using daily) users (AN / smokers/ alcohol) and chewers using AN for less than 6 months were not included in the study.

After obtaining informed verbal consent, case history followed by careful oral examination was done for all subjects. Details of the chewing habit of the subjects were recorded which included the type of chewing material along with the details of frequency per day (≤ 5 times, > 5 times), duration (≤ 5 years, > 5 years), quantity and exposure time (≤ 5 minutes, > 5 minutes) were noted down. In case of smokers the duration and frequency were recorded. For smokers, pack years (in terms of 10’s pack) were calculated by the approximate number of sticks smoked per year divided by 10 and intensity is the approximate number of sticks smoked till date. Alcohol habit was recorded as regular users or non-users. For the purpose of the study the AN chewers were divided into RAN, PAN, BQT and BQ.

Saliva Collection

Saliva collection was done between 9:00 am to 12:00 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 entire study. Subjects were then seated in the dental chair and asked to spit in a graduated container every 1-minute for 10 minutes. During saliva collection subjects were instructed not to speak or swallow. After collection the SFR was measured and expressed in mL/10 minutes.

Salivary pH was measured immediately after measuring SFR using the Dental Salivary pH Indicator (pH 5.0-8.0, Saliva check buffer Kit, GC Corporation, Tokyo, Japan). Based on the color change of the indicator paper strip, the pH was assessed in comparison with a color chart. Similar methods to assess the buffering capacity of saliva have been shown to be valid. Manufacturer’s instructions were followed while measuring salivary pH. The whole mouth resting saliva collected after measurement is taken in a watch glass and the indicator strip is dipped in the saliva for 30 seconds and the color on the strip compared with the standard color chart provided by manufacturer. The corresponding value is taken as the salivary pH.

Statistics

Data were entered and analyzed using Statistical Package for Social Services, Version 10.0.5. Student t test was employed to find the statistical significance of difference in mean SFR and mean pH between chewers and non-chewers. Student t test was utilized to find the mean difference in SFR and pH in duration, intensity and frequency among the various types of AN. The Spearman’s correlation coefficient was employed to find the correlation between pack years, intensity with SFR and pH and also between SFR and pH in chewers and non-chewers who smoke cigarettes. The mean SFR and pH of chewers and non-chewers, depending on their smoking and alcohol use status were done and student t test used to find the mean difference in the values. A p value of less than 0.05 was considered as statistically significant. 95% confidence interval, test statistic value and degree of freedom were given for the entire student t tests performed.

Result

The study group comprised of 110 chewers and 50 non-chewers as non-chewers. There were 91 males and 19 females among chewers and 43 males and 7 females among
non-chewers. The age distribution of the study population is given in [Table 1]. It was observed that 48.19% of chewers were in the age group of 21 to 30 years. The most commonly used form of AN was PAN (68%), BQT (14%), RAN (10%) and BQ (8%).

The mean frequency, exposure time and duration of chewing habit are listed in [Table 2]. The mean SFR for chewers was 3.35 ± 1.7 and for non-chewers it was 3.55 ± 1.39. The difference was not statistically significant. (p=0.5) The pH of chewers was 6.57 ± 0.52 and for non-chewers it was 6.77 ± 0.41. The difference was statistically significant (p=0.02). [Table 3] The correlation coefficient between SFR and pH in non-chewers was 0.27 (p=0.06) while in chewers it was 0.44 (p<0.001) [Figure 1]. The RAN chewers had a mean SFR of 4.18 ± 2.5 and pH of 6.49 ± 0.7, while PAN chewers had 3.32 ± 1.57, 6.64 ± 0.5, BQT chewers had 3.37 ± 1.23, 6.47 ± 0.46 and BQ chewers 2.56 ± 1.7, 6.27 ± 0.51 respectively [Figures 2 and 3].

The type of AN was analyzed for its influence on SFR [Table 4] and salivary pH [Table 5]. It was observed that with chewing RAN, an increase in frequency of consumption and exposure time increased SFR (p=0.005) and pH (p=0.005) respectively. In PAN chewers, increase in duration and frequency of consumption increased the SFR (p=0.06) and decreased the pH (p=0.011) respectively. For BQT chewers, increase in duration of the habit was significantly associated with the decrease in salivary pH (p=0.04). For BQ, the difference in SFR and pH was not statistically significant with respect to the frequency, duration or exposure.

There were 4 smokers among non-chewers with a mean SFR of 3.88 ± 1.32 while the mean SFR non-smokers was 3.52 ± 1.41. There were 21 smokers among chewers with a mean SFR of 3.12 ± 1.56 while the mean SFR of non-smokers was 3.40 ± 1.69. There were 5 alcohol users among non-chewers with a mean SFR 3.9 ± 1.67 while the mean SFR of non-alcohol users was 3.51 ± 1.38. There were 11 alcohol users among chewers with a mean SFR of 2.68 ± 0.98 while the mean SFR of non-alcohol users was 3.42 ± 1.71. The difference in mean SFR was not statistically significant between chewers and non chewers as well as between smokers and non smokers as also in alcohol users and non alcohol users. [Table 6]

The non-smokers who chewed had a salivary pH of 6.59 ± 0.56 while non-chewers had 6.77 ± 0.43. The difference was statistically significant. (p=0.039) The alcohol users who chewed AN had a mean pH of 6.26 ± 0.53 while those did not chew AN had 6.92 ± 0.27. The difference was statistically significant. (p=0.005). Non-alcohol users who chewed AN had a pH of 6.60 ± 0.5. The difference between the mean pH of AN chewers who used alcohol and those who did not use alcohol was statistically significant. (p=0.036) [Table 7]

Spearman’s Rho correlation was applied for pack years, intensity, SFR and pH among chewers, pack years had a correlation of 0.416 with borderline statistical significance.
Table 4: Mean salivary flow rate in each type of arecanut

<table>
<thead>
<tr>
<th>Type of AN</th>
<th>Parameters</th>
<th>n</th>
<th>Salivary flow rate Mean ± SD</th>
<th>95% CI</th>
<th>t value</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAN</td>
<td>Duration</td>
<td>≤5 years</td>
<td>6</td>
<td>3.17 ± 1.97</td>
<td>1.10 - 5.23</td>
<td>-1.58</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>&gt;5 years</td>
<td>5</td>
<td>5.4 ± 2.73</td>
<td>2.02 - 8.78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frequency per day</td>
<td>≤5 times</td>
<td>10</td>
<td>3.6 ± 1.68</td>
<td>2.40 - 4.80</td>
<td>-3.63</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;5 times</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Exposure per use</td>
<td>≤5 minutes</td>
<td>8</td>
<td>3.56 ± 1.86</td>
<td>2.01 - 5.12</td>
<td>-1.40</td>
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<td></td>
<td></td>
<td>&gt;5 minutes</td>
<td>3</td>
<td>5.83 ± 3.69</td>
<td>-3.32 - 14.99</td>
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<td></td>
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<tr>
<td>PAN</td>
<td>Duration</td>
<td>≤5 years</td>
<td>56</td>
<td>3.53 ± 1.31</td>
<td>2.97 - 4.34</td>
<td>-1.88</td>
<td>73</td>
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<tr>
<td></td>
<td>&gt;5 years</td>
<td>19</td>
<td>3.9 ± 2.1</td>
<td>2.88 - 4.91</td>
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<td></td>
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<tr>
<td></td>
<td>Frequency per day</td>
<td>≤5 times</td>
<td>63</td>
<td>3.29 ± 1.61</td>
<td>2.88 - 3.69</td>
<td>-0.43</td>
<td>73</td>
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<td></td>
<td></td>
<td>&gt;5 times</td>
<td>12</td>
<td>3.5 ± 1.47</td>
<td>2.53 - 4.51</td>
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<tr>
<td></td>
<td>Exposure per use</td>
<td>≤5 minutes</td>
<td>30</td>
<td>3.43 ± 1.63</td>
<td>2.83 - 4.04</td>
<td>0.51</td>
<td>73</td>
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<tr>
<td></td>
<td></td>
<td>&gt;5 minutes</td>
<td>45</td>
<td>3.24 ± 1.54</td>
<td>2.78 - 3.71</td>
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<tr>
<td>BOT</td>
<td>Duration</td>
<td>≤5 years</td>
<td>12</td>
<td>3.54 ± 1.23</td>
<td>2.76 - 4.33</td>
<td>1.11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>&gt;5 years</td>
<td>3</td>
<td>2.67 ± 1.16</td>
<td>2.0 - 5.54</td>
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<td></td>
<td>Frequency per day</td>
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<td>11</td>
<td>3.32 ± 1.27</td>
<td>2.47 - 4.17</td>
<td>-0.24</td>
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<td></td>
<td>&gt;5 times</td>
<td>4</td>
<td>3.5 ± 1.29</td>
<td>1.45 - 5.55</td>
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<tr>
<td></td>
<td>Exposure per use</td>
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<td>9</td>
<td>3.61 ± 1.22</td>
<td>2.67 - 4.55</td>
<td>0.94</td>
<td>13</td>
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<td></td>
<td>&gt;5 minutes</td>
<td>6</td>
<td>3 ± 1.27</td>
<td>1.67 - 4.33</td>
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<td></td>
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<tr>
<td>BO</td>
<td>Duration</td>
<td>≤5 years</td>
<td>4</td>
<td>2.38 ± 1.49</td>
<td>-0.007 - 4.75</td>
<td>0.27</td>
<td>7</td>
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<tr>
<td></td>
<td>&gt;5 years</td>
<td>5</td>
<td>2.7 ± 2.02</td>
<td>-0.19 - 5.23</td>
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<td></td>
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<tr>
<td></td>
<td>Frequency per day</td>
<td>≤5 times</td>
<td>8</td>
<td>2.81 ± 1.62</td>
<td>1.46 - 4.17</td>
<td>1.34</td>
<td>7</td>
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<td>0.5</td>
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<tr>
<td></td>
<td>Exposure per use</td>
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<td>2.4 ± 2.19</td>
<td>-0.79 - 4.01</td>
<td>-0.29</td>
<td>7</td>
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<tr>
<td></td>
<td></td>
<td>&gt;5 minutes</td>
<td>4</td>
<td>2.75 ± 2.33</td>
<td>-0.95 - 6.45</td>
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</tr>
</tbody>
</table>

*P = 0.05.

Numbers are too small to infer results. SD is not there due to single case.

SD – Standard deviation; CI - confidence interval; t – Test value; df – degree of freedom

RAN – raw arecanut; PAN – processed arecanut products; BQT – arecanut, betel leaves, lime, tobacco and condiments; BO – arecanut, betel leaf, lime and condiments

Salivary flow rate: measured in mL/10 minutes.

with SFR. (p=0.061) while intensity had a correlation of 0.58 (p=0.006) with SFR and a negative correlation with pH (r = -0.53) but without statistical significance.

**DISCUSSION**

The effects of AN chewing are habit related and dose dependent.[19] The report of effects being more pronounced in fresh or occasional chewers and less in habitual chewers, suggests that the tolerance or habituation also occurs in AN use.[19] Hence habituation to the stimulus occurs in the receptors.[10]

RAN has highest mean SFR (4.18 mL/10 min) as compared to the non-chewers (3.55 mL/10 min) and other chewers. This is probably due to the parasympathomimetic activity of arecoline. In BO the mean SFR drops to the lowest (2.56 mL/10 min) probably due to lime that converts arecoline to arecadine. While in BQT the mean SFR is increased (3.37 mL/10 min). In case of PAN there was an increase in SFR (3.32 mL/10 min) as compared to SFR of those who chew BQT. This phenomenon could be due to the interaction of various components of quid with tobacco. This is consistent with observations of Jenner at al, 1973 who observed that people, who chew tobacco, generate large amounts of saliva.[15] It has been reported by Khan GJ, 2003 that long term use of tobacco does not adversely alter the SFR.[12]

Among those who chewed RAN, the difference in mean SFR between frequency of chewing (p=0.005) and in those who used PAN, duration of chewing habit (p=0.06) had a statistical significance. It is reported that SFR in BQT chewers were comparatively higher and significant than non-chewers.[19] It is suggested that this might be due to increased salivary gland mass as a result of chronic chewing or due to chronic exposure to one or all of the constituents of BQT quid and chronic BQT chewing induces excessive secretion of more watery saliva leading to a concomitant decrease in enzyme, electrolyte content and altered pH.[11,12] The coarse BQT will require more masticatory forces that also increase SFR.[16] However it has been reported by Khan GJ, 2003 that long term use of tobacco does not adversely alter the SFR.[12]

The mean pH of non-chewers was 6.77. In those who chewed RAN, the mean pH turns acidic (6.49), as the secretion is seriously probably with more secretion of sodium ions as described by Khan GJ, 2003.[13] An increase in SFR alters salivary pH by increasing bicarbonate secretion.[13] An increase in saliva bicarbonate increases the pH.[20] In habitual BQ chewers, lime probably reacts with bicarbonate buffering system by the loss of bicarbonate, turning saliva more acidic (6.27). In those who chew BQT and
PAN, the SFR decreases turning the pH less acidic to 6.47 and 6.64 respectively. Even though the difference in values are statistically significant (p=0.024), the small sample size and unequal distribution may be a limitation. In contrast Reddy MS et al, 1980, observed no difference in salivary pH between the chewers and non-chewers. This difference could be due to the amount of tobacco, lime and other components. The role of lime in PAN, BOT and BO has been a source of concern. Lime (calcium oxide in aqueous forms calcium hydroxide) could cause a free radical injury or the high alkali content probably reacts with the salivary buffering systems and alters the pH. Formation of reactive oxygen species in the oral cavity during betel-quid chewing has been demonstrated. In-vitro studies have shown that the generation of reactive oxygen species is due to auto-oxidation of the polyphenols in areca nut and catechu. This reaction is enhanced by alkaline pH and by the presence of the transition metals, copper and iron.

SFR influences the pH of saliva. Studies using gum have shown that an increase in mastication, in normal subjects enhances the bite force as well as the SFR. In case of chewers using coarser RAN, there is increased SFR as it requires more masticatory force.

The statistical significant correlation between SFR and pH in chewers shows an increasing pattern may reflect an alteration in the electrolyte constituent of saliva in chewers. The alteration in electrolytes and ions alters the pH as they interact with the buffering systems of saliva. The marked changes with alcohol use indicate that with concomitant use, SFR and pH are altered to a greater extent.

Our observations are based on this preliminary study, in which the sample size was small with unequal distribution in between genders and among types of AN, multiplicity of factors and SFR and pH varying with a wide range are limitations of the study. This study is the first to our best of knowledge to site the SFR and pH in AN chewers and one to point the difference in SFR and pH between various types of AN. We consider further analyses like: amount of active compounds released difference in SFR and pH between various types of AN. We consider further analyses like: amount of active compounds released from AN during chewing and also those that are absorbed into the circulation and the brain, possible complex interactions between various absorbed active compounds in the brain and the autonomic nervous system, the biological in-equivalence of all components of AN products and the sensitization of receptors and habitation with chronic chewing in a larger sample will reveal processes involved in AN chewing and their effect on SFR and pH.

**CONCLUSION**

Alterations in SFR and salivary pH are observed in habitual AN chewers. The alteration is dependent on the type of AN chewed. The alteration in SFR and pH are vital in causation of various oral diseases. Moreover, the complex action of AN chewing is also reflected as variation in SFR and pH.

**ACKNOWLEDGEMENT**

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**REFERENCES**

We report a patient with strongyloidiasis in patients with ulcerative colitis. Patients with severe ulcerative colitis who are immunosuppressed may develop fatal hyperinfection with this nematode. Patients with severe ulcerative colitis who are immunosuppressed may develop fatal hyperinfection with this nematode. Patients with severe ulcerative colitis who are immunosuppressed may develop fatal hyperinfection with this nematode. Patients with severe ulcerative colitis who are immunosuppressed may develop fatal hyperinfection with this nematode.

**References**