Characterization of Saliva in Immunocompromised Patients and Tobacco Users: A Case–Control Study

Abstract

Objective: The aim of the study was to assess salivary flow rate, salivary pH, and salivary albumin concentration in systemically compromised subjects and tobacco users and its comparison to healthy controls.

Materials and Methods: Sixty patients were selected and then were equally divided into systemically compromised group, tobacco users, and control group with 20 in each group. Saliva flow rate was assessed using modified Schirmer test (MST), salivary pH was estimated by pH meter, and salivary albumin concentration was determined using bromocresol green method.

Results: The salivary flow rate readings measured by MST were 22.65 ± 2.79, 22.6 ± 3.57, and 33.22 ± 2.30 mm/3min in systemically compromised individuals, tobacco users, and control group, respectively (P < 0.001). The salivary pH was 6.80 ± 0.24, 6.81 ± 0.25, and 7.18 ± 0.17 in systemically compromised subjects, tobacco users, and control group, respectively (P < 0.001). The salivary albumin concentration was 2.49 ± 0.61, 0.73 ± 0.13, and 1.14 ± 0.12 g/dl in systemically compromised subjects, tobacco users, and control group, respectively (P < 0.001).

Conclusion: MST can be routinely used as chair-side investigation to evaluate salivary flow which is objective, inexpensive, easy-to-perform, and patient-friendly. The salivary flow rate, salivary pH, and salivary albumin level in systemically compromised subjects, tobacco users, and normal individuals showed significant differences.

Keywords: Modified Schirmer test (MST), saliva, salivary albumin, salivary flow, salivary pH

Introduction

Saliva is crucial for the maintenance of oral and systemic homeostasis. Salivary hypofunction predisposes patients to disorders such as dysgeusia, pain and burning mouth, caries and other oral infectious diseases, dysphagia, and dysphonia. Decreased salivation is one of the most common side effects of certain drugs. It has been reported that 29% of the adult population suffers from xerostomia or have subjective complaint of dry mouth. Several studies have associated medications with decreased salivary flow.

Long-term use of tobacco affects the sensitivity of taste receptors adversely which in turn leads to diminished salivary reflex. This might probably lead to altered taste receptors’ response and thus result in impaired salivary flow rate.

The pH of saliva is maintained by carbonic acid/bicarbonate, phosphate, and protein system. It is also monitored by sympathetic and parasympathetic regulation of salivary flow. Tobacco usage immediately stimulates salivary flow, but long-term effect on salivary flow rates is still questionable. In the presence of lime, arecoline and guvacoline are hydrolyzed mostly into arecaidine and guvacine, respectively.

Salivary albumin is considered as a serum ultrafiltrate and it may diffuse into oral mucosal secretions. Salivary total protein is undoubtedly a very important component of saliva maintaining the integrity of mucosal function in the mouth, predominantly comprising proline-rich proteins. Salivary albumin is increased in medically compromised patients like immunosuppression, radiotherapy, and diabetes.

Quantitative and qualitative salivary changes associated with local or systemic disorders are not always easily diagnosed by clinicians and scientists owing to the lack of standardization in saliva collection methods.

Numerous efforts have been made to develop a method for salivary flow...
determination which are reproducible, patient-compatible, less time-consuming, and minimally cumbersome. One such new alternative is the use of Schirmer tear test strips which are easily commercially available and routinely used by ophthalmologists to measure tear gland function. Till date, only three studies have been reported, where saliva secretion was measured using paper strips in a manner similar to Schirmer’s test.

The aim of this study was to assess the salivary flow rate in systemically compromised subjects and tobacco users by modified Schirmer test (MST) and its comparison to control group. In addition, the study also aims to assess and compare the salivary pH and salivary albumin concentration in the same group of patients.

Materials and Methods

This study was carried out on outpatients visiting the Department of Oral Medicine and Radiology of a dental college during the period of August–October 2015. Informed consent was obtained from each subject included in the study. Ethical approval was obtained from the institutional review board of the college on 212/2015-16).

Inclusion criteria

• Having systemic disease for at least 1 year duration
• On medication for systemic disease
• Having the habit for tobacco use for the past 1 year.

Exclusion criteria

• History of radiotherapy of head and neck
• Having any oral mucosal lesions or salivary gland pathology
• Pregnancy
• Having overlapping systemic disease and tobacco habit.

Sixty patients were randomly selected according to inclusion criteria. These patients were equally divided into the following groups:

Group I: systemically compromised (hypertension and diabetic)

Group II: tobacco users

Group III: control (healthy individuals).

Determination of salivary flow rate

An unstimulated salivary flow was measured using an MST. The commercially available 5 × 35 mm Color Bar Schirmer tear test strip (Madhu Instruments, Delhi, India) has a blue color bar that travels with the fluid through its length (0–35 mm) denoting the amount of fluid flow.

Subjects were asked to sit in dental chair in an upright position. They were then instructed to swallow all the residual saliva in the mouth prior to the test and to rest the tongue on the anterior hard palate so that the tongue does not touch the Schirmer tear strip while performing the test. They were also advised not to swallow anymore during the test. The MST strip was held vertically by a tweezer at the straight end in such a way that the tip of the rounded end touched the floor of the mouth either on the right or the left of the lingual frenum [Figure 1]. On contact with the saliva/moisture, the blue dye traveled upward. The reading was recorded accordingly at the end of the third minute [Figure 2]. In this study, hyposalivation was assessed if the color moved <25 mm at 3 min as suggested by Fontana et al. [9]

Collection of unstimulated saliva

Unstimulated saliva collection was done for salivary albumin and pH determination. It was done in the morning hours between 9 am and 12 noon. All the patients were asked to refrain from eating, drinking, and chewing tobacco for at least 90 min before the proceedings. Unstimulated saliva was collected by spitting method where the patient was asked to spit in a preweighed container every 60 s for 10 min. [10]

Salivary pH was estimated with the help of a pH meter electrometrically. A pair of electrodes was dipped in the container containing saliva [Figure 3]. A potential difference developed across the thin glass of the bulb (electrode). Salivary pH varied in accordance to electromotive forces (E). These variations were recorded directly on the graduated scale to read pH directly.

The salivary albumin concentration estimation was done using bromocresol green (BCG) dye method [Figure 4]. Albumin binds with BCG dye at pH 4.2. The reaction between albumin and BCG leads to a shift in absorbance of the yellow BCG dye to blue-green. This blue green coloration formed is proportional to the concentration of albumin present. Thus, by measuring photometrically between 580 and 630 nm with a maximum absorbance at 625 nm, the albumin concentration can be estimated. [11] All albumin estimations were performed using reagent kits of Transasia Bio-Medical Ltd. (ERBA, Mannheim, Germany), using an Autoanalyzer (ERBA).
Statistical analysis

Salivary flow rate, salivary albumin level, and pH were estimated by one-way analysis of variance (ANOVA), followed by Tukey’s post hoc test using SPSS (version 17.0). A P value ≤0.05 was considered statistically significant.

Results

Our study included 60 individuals, 20 in each of three groups. Demographic data showed that group I consisted of 14 males and 6 females (hypertensive: 12, diabetic: 8) with age range of 36–59 years (mean age = 44.7 years), group II had 13 males and 7 females with age range of 32–57 years (mean age = 40.95 years), and group III included 13 males and 7 females with age range of 32–59 years (mean age = 42.65 years).

On applying one-way ANOVA test, it was inferred that salivary flow for group I was 22.65 ± 2.79 mm/3min, for group II was 22.6 ± 3.57 mm/3min, and for group III was 33.22 ± 2.30 mm/3min.

Along with this, salivary pH was also determined for all the patients. It was seen that salivary pH for group I was 6.80 ± 0.24, for group II was 6.81 ± 0.25, and for group III was 7.18 ± 0.17.

In addition, another parameter, that is, salivary albumin concentration was also calculated for group I to be 2.49 ± 0.61 g/dl, for group II 0.73 ± 0.13 g/dl, and for group III 1.14 ± 0.12 g/dl.

For all the three parameters, the P value was less than 0.001, that is, statistically highly significant [Table 1].

After applying Tukey’s post hoc test to the values obtained from one-way ANOVA test, it was observed that salivary flow was greatest in the control group (group III). However, it was comparable in groups I and II with a P value <0.001, that is, highly significant (III >I, II). In relation to salivary pH, group III has shown the most alkaline pH when compared with groups I and II with a P value <0.001, that is, highly significant (III >II, I). And in context to salivary albumin level, it was greatest in group I and least in group II with a P value <0.001, that is, highly significant (I>III>II) [Graph 1].

Discussion

Salivary flow rate and its constituents play a crucial role in the maintenance of integrity of oral cavity including soft and hard tissues. To the best of our knowledge and extensive literature search, our study is the third to use MST for assessment of salivary flow in systemically compromised and tobacco users.

Although hypertension and diabetics as systemic compromised conditions are studied extensively, their effect on salivary constitution and functions is still undiscovered. In this study, salivary flow, pH, and albumin concentration were also determined separately for hypertensive and
diabetic individuals. The difference between the values among the hypertensive and diabetic individuals were not significant.

This study revealed a reduction in salivary flow among study group I (22.65 ± 2.79 mm/3min) when compared with control group III (33.22 ± 2.30 mm/3min), and the difference was statistically highly significant (P < 0.001). The reduction in salivary flow rate in hypertension can be explained on the basis of decrease in blood flow to salivary glands as a part of homeostasis regulation by rennin angiotensin system. Secretion of angiotensin II results in vasoconstriction that finally leads to a decrease in blood flow to salivary glands, thus impairing its function.[13]

The reduction in the salivary flow rate may lead to reduction in the salivary constituents which include the salivary buffer, and this can result in a low/acidic pH.[14]

Salivary albumin level was found elevated in hypertension through β-sympathetic activity of salivary glands, since saliva secretion is mainly evoked by the action of adrenergic mediator.

Also, a decrease in salivary flow rate was observed in diabetic patients, which can be explained on the basis of polyuria resulting in an extracellular dehydration. This in turn results in decreased blood flow to the salivary gland, thus finally leading to a decrease in salivary flow.[15]

This study demonstrated that when the diabetic individuals were compared with the control group (7.18 ± 0.17), the diabetics had decreased salivary pH values (6.8 ± 0.24). Presumably, it may be due to the changes in the metabolic processes of the diabetics due to higher glucose levels, resulting in a more acidic environment.[16]

We also found an increase in salivary albumin concentration in diabetic patients (2.49 ± 0.61 g/dl), and our results are in agreement with the reports of Ben-Arch[17] and Dodd et al.[18] Typically, albumin, as a serum protein, is substantially increased, like lactoferrine, with acute inflammation of the salivary glands. This suggests that low-grade infection of the salivary glands causes increased leakage of serum proteins into the saliva which is a common finding in diabetic patients.[19] Although Meurman et al.,[19] Carda et al. [20] and Collin et al. [21] reported that there was no difference found in salivary albumin between diabetic patients and control group.

Several studies on the estimation of unstimulated salivary flow conducted among healthy individuals concluded 0.3–0.5 ml/min as normal state of functioning, whereas <0.1 ml/min is considered as hyposalivation. In this study, the mean salivary flow rate determined by MST among tobacco users (22.60 ± 3.57 mm/3min) was decreased when compared with control group (33.22 ± 2.30 mm/3min).

Contradictory to the results of our study, Rooban et al. [22] and Siddabasappa et al. [23] observed an increase in salivary flow rate in tobacco chewers to be 0.418 and 0.61 ± 0.07 ml/min, respectively.

Whereas Kanwar et al. [24] found a decrease in salivary flow rate in long-term tobacco chewers. Rad et al. [25] also observed a significant decrease in salivary flow rate in chronic smokers.

Tobacco can result in parasympathetic stimulation of post-ganglionic neurons in response to nicotine, in a manner similar to acetylcholine, because the membrane of these neurons contains nicotinic type of acetylcholine receptors. This can be the cause for increased salivation during chewing period, but in the absence of tobacco chewing period salivary flow can decrease. Previous literature also mentions that nicotine can cause alteration in autonomic nervous system by increasing plasma level of epinephrine and norepinephrine which results in a decrease in salivary flow rate.[26]

It has also been suggested that a decrease in salivary flow rate can be due to the effect of nicotine over taste receptors.

**Table 1: Salivary flow rate, salivary pH, and salivary albumin concentration in groups I, II, and III, respectively**

| Systemically compromised (I) | Salivary flow (mm/3min) | Salivary pH | Salivary albumin (g/dl) |
|-----------------------------|-------------------------|-------------|------------------------|
| Tobacco users (II)          | 22.6±2.79               | 6.8±0.24    | 2.49±0.61              |
| Control (III)               | 33.22±2.30              | 7.18±0.17   | 1.14±0.12              |

Results

- **III>I, II**
- **III>II, I**
- **I>III>I**

**P<0.001** indicates highly significant difference exists between the groups

**Graph 1: Bar graph representation of the salivary flow rate, salivary pH, and salivary albumin concentration in systemically compromised tobacco users and control group**
by depressing salivary reflex or degeneration of salivary glands.

Rooban et al.\cite{22} and Kanwar et al.\cite{24} have found acidic pH among tobacco chewers similar to our study \((6.81 \pm 0.25)\). It can be suggestive of lime used by chewers reacting with the buffering system of saliva and decreasing the bicarbonate level, thus converting pH into acidic.

Contrary to our results, Siddabasappa et al.\cite{23} and Reddy et al.\cite{27} did not find any significant change in salivary pH of tobacco users and healthy control.

In our study, salivary albumin level in tobacco users \((0.73 \pm 0.13 \text{ g/dl})\) was low when compared with the control group \((1.14 \pm 0.12 \text{ g/dl})\). This can be explained on the basis that nicotine results in parasympathetic stimulation which leads to production of saliva with little protein.

**Limitations**

Several limitations of this study should be considered while interpreting the results. First, all the important parameters were not evaluated for the characterization of saliva, such as total salivary protein, viscosity, amylase, and electrolyte concentration. Second, gingival and periodontal status was not considered along with decayed, missing, and filled teeth (DMFT) index. These factors are very crucial in altering the salivary composition and flow. Third, age and sex of the patient were not taken into account, which were also important factors related to salivary flow and constitution. Fourth, the sample size was small.

Therefore, future studies could be done with larger sample size as well as different groups of patients. This will help in determining specific characteristics of saliva in different types of xerostomic patients.

Furthermore, research can also be carried to determine the other components of the saliva that are altered in different pathologic conditions and systemic conditions. This study leads a path for development of various customized salivary substitutes which are patient-specific and thus help oral clinicians treat xerostomia in a better way.

**Conclusion**

MST used in this study is inexpensive, reproducible, less time-taking, least cumbersome to perform, and well-tolerated by patients. It can be routinely used as a chair-side investigation to distinguish between patients who are healthy and xerostomic patients. This study showed that there is a significant correlation between salivary flow and its components with the systemic status of the body and habit of tobacco. This study is also a stepping stone for determining the type of salivary substitute required for a particular group of xerostomic patients.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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