Original Article

A relationship between salivary flow rates and Candida counts in patients with xerostomia

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Abstract

Context: Most of the adult population is colonized by Candida in their oral cavity. The process of colonization depends on several factors, including the interaction between Candida and salivary proteins. Therefore, salivary gland hypofunction may alter the oral microbiota and increase the risk for opportunistic infections, such as candidiasis. Hence, it is necessary to evaluate the relationship between salivary flow rates (SFRs) and Candida colony counts in the saliva of patients with xerostomia.

Aims: This study aims to determine and evaluate the relationship between SFRs and Candida colony forming units (CFUs) in patients with xerostomia.

Settings and Design: This study was a descriptive study.

Subjects and Methods: The study participants were taken from the patients attending outpatient department in a private dental college. Fifty patients, who reported xerostomia in a questionnaire of the symptoms of xerostomia, were selected. Chewing stimulated whole saliva samples were collected from them and their SFRs were assessed. Saliva samples were inoculated in the Sabouraud dextrose agar culture media for 24–48 h, and Candida CFUs were counted.

Statistical Analysis Used: Chi-squared test was used to analyze the data.

Results: There was a significant inverse relationship between salivary flow and candida CFUs count when patients with high colony counts were analyzed (cutoff point of 400 or greater CFU/mL). Females had less SFR than males. Most of the patients who had hyposalivation were taking medication for the underlying systemic diseases. *Candida albicans* was the most frequent species.

Conclusions: There was a significantly negative correlation between SFRs and Candida CFUs in the patients with xerostomia.

Keywords: Candida colony forming units, medications, salivary flow rates, salivary gland hypofunction, xerostomia

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INTRODUCTION

Saliva plays a crucial role in oral health. It buffers acids, has antibodies, helps prevent gingival mucosal erosions and ulcerations and aids in tooth remineralization. When salivary function is diminished, there is more risk of patients developing caries, experiencing denture discomfort and having diseases such as candidiasis than in patients who have normal salivary flow rates (SFRs).[1]

Xerostomia or dry mouth is defined as the subjective feeling of oral dryness perceived by the patient. The term hyposalivation is the reduction in SFR and it is an objective sign.[2]

Hyposalivation is the most common etiologic factor in xerostomia. Hyposalivation may be caused by different etiologic factors. They are developmental abnormalities of salivary glands, age, systemic diseases, medications and radiotherapy.[3]

Salivary gland hypofunction may alter the oral microbiota and increase the risk of oral candidiasis, which is the most prevalent opportunistic infection affecting the oral mucosa, caused by Candida species.[4]

Sixty percent of healthy adults and 45%–65% of healthy children may harbor commensal Candida microbe without any clinical signs and symptoms.[5]

Under variety of pathological conditions, Candida can proliferate in the mouth and produce oral lesions. This correlation is more significant in patients with medical conditions such as Sjogren's syndrome, diabetes mellitus, cardiovascular diseases, psychological disorders, thyroid dysfunction, hepatitis C infection and human immunodeficiency virus (HIV) infection.[5]

Saliva plays a significant role in oral homeostasis. It contains antimicrobial proteins such as lysozyme, lactoperoxidase, immunoglobulins, histatins and lactoferrin. Histatins have potent antifungal activity and there is also some evidence that salivary IgA inhibits oral adhesion of *Candida albicans*. The salivary calcium-binding myelomonocytic L1 protein or calciprotein also play a role in the defense against oral candidiasis in HIV-infected patients.[5]

With the increasing numbers of geriatric and medically compromised patients being seen by dental practitioners and oral diagnosticians, longitudinal evaluation of whole saliva should be considered as part of the clinical evaluation to identify patients at risk for candidiasis and prevent clinical complications.[4]

The current study is therefore undertaken to determine and evaluate the relationship between SFRs and Candida colony forming units (CFUs) in patients with xerostomia.

SUBJECTS AND METHODS

Source of data

The study participants were taken from the patients attending the outpatient department of a private dental college. Randomly selected outpatients were asked to answer a questionnaire with a list of symptoms associated with xerostomia. Fifty patients who answered affirmatively to few of the questions in the questionnaire were included in the study.

Questionnaire used for the selection of patients with xerostomia:

1. Does your mouth feel dry when eating a meal?
2. Do you have difficulty in swallowing any food?
3. Do you sip liquids to aid in swallowing dry foods?
4. Does the amount of saliva in your mouth seem to be reduced most of the time?
5. Does your mouth feel dry at night or on awakening?
6. Do you chew gum or use candy to relieve oral dryness?
7. Does your mouth feel dry during daytime?
8. Do you usually wake up thirsty at night?
9. Do you have problems in tasting food?
10. Does your tongue burn?

Patients who had been on corticosteroids and/or antibiotics within previous 3 months were excluded from the study.

Ethical clearance

The study protocol was approved by Institutional Ethical Committee. Patients were given both verbal and written information about the nature of the study, and written consent was obtained.

Method of collection of data

Saliva collection method

For all the selected patients, intraoral clinical examination was done to record any features of xerostomia.

Samples of chewing-stimulated whole saliva were obtained under standard conditions.

Patients were asked not to eat, drink (water exempted), smoke, or perform any oral hygiene habits 1 h before saliva collection. Patients were asked to chew sugarless gum for 5 min. They were instructed to spit the accumulated saliva after every minute into a sterile graduated container [Figure 1]. Only the liquid component of the saliva was measured.

SFRs were determined by milliliter/minute. Hyposalivation was considered if SFR was <1 ml/min.

Collected saliva samples were transported in ice packs to the laboratory and inoculated within 2 h.

Culture method

In the laboratory, saliva samples were centrifuged for 2 min. 0.1 ml of that saliva was inoculated on Sabouraud dextrose chloramphenicol agar culture plates and incubated at 37° Celsius for 24–48 h. The creamy white-colored, smooth colonies with yeasty odor were suggestive of Candida growth [Figure 2].

After 48 h, the colonies so formed were counted as number of CFUs/ml.

A cutoff point was established for colony counts so that 400 or greater CFU/ml was considered as a high CFU count.

Colonies formed were smeared on the slide. Gram staining was done for the smear, which showed violet-colored budding yeast like fungus suggesting Gram positive.

The discrete colonies were selected and subcultured in Sabouraud dextrose agar culture tubes. Candidal growth from the subculture tubes was later inoculated on the HiCrome Candida differential agar and incubated at 37°C for 24–48 h for identification of Candida species. Different Candida species produce different colored colonies on HiCrome Candida differential agar [Figure 3]. *C. albicans* produces green-colored colonies, *Candida tropicalis* produces blue-colored colonies and Candida krusei produces pale rose-colored colonies.

Statistical analysis

All the findings were tabulated and the results were analyzed using Chi-squared test.

RESULTS

Out of fifty patients, 16 (34%) were males and 34 (68%) were females. Mean overall age of the patients is 53.94 years, mean age in males is 57.50 years and mean age in females is 52.26 years.

Patients with <1 ml/min of SFR were considered to have hyposalivation and patients with >1 ml/min of saliva flow rate were considered to have normal salivation. Among fifty patients, 26 (52%) had normal salivation and 24 (48%) had hyposalivation.

Out of fifty samples, 32 (64%) samples showed Candida colonies and 18 (36%) samples did not show any Candida growth.

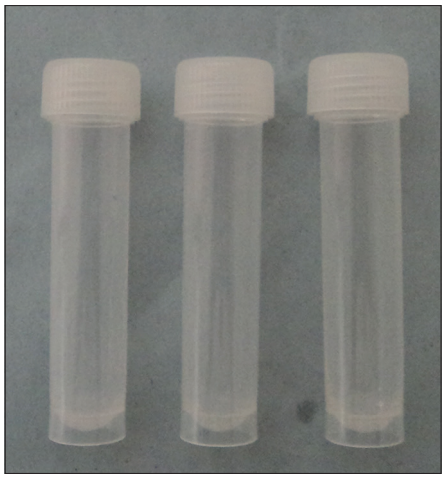

Figure 1: Sterile graduated plastic tubes for collection of saliva

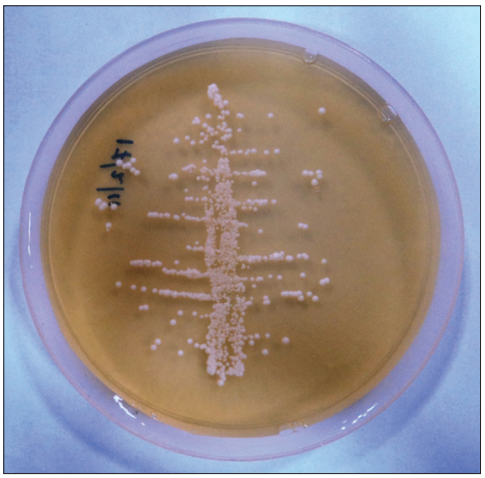

Figure 2: Culture plate with Sabouraud dextrose agar media showing Candida colonies

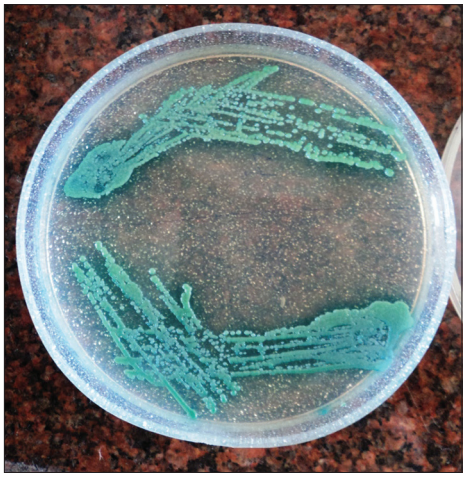

Figure 3: Culture plate with Chromagar media showing green-colored *Candida albicans* colonies

To know the number of Candida colonies found among fifty samples, a cutoff point was established for colony counts so that 400 or greater CFU/ml was considered as a high CFU count. Twenty (40%) samples showed more than or equal to 400 CFU/ml of saliva and 30 (60%) samples showed less 400 CFU/ml of saliva. Those samples which showed ≥400 CFU/ml were reinoculated in HiCrome Candida differential agar media for Candida species identification.

Type of Candida species formed among twenty samples which got ≥400 CFU/ml were evaluated. Out of twenty samples, 17 (85%) were *C. albicans* (green color colonies) and 3 (15%) were *C. tropicalis* (blue-colored colonies).

Out of fifty patients, 34 (68%) were medically compromised and were under one or many medications for the same. Sixteen (32%) were without any medication.

Relationship between salivation and Candida colony formation has been tabulated in Table 1. Statistically significant association is observed between Candida colonies formation and salivation ($P < 0.001$).

Relationship between salivation and number of Candida CFUs formed has been tabulated in Table 2. Statistically significant association is observed between colony formation and salivation ($P < 0.001$).

With the above results, we can infer that symptoms of xerostomia were more in female patients and also in those patients who were taking one or more medications for systemic illness. We also observed that salivation and Candida colony formation were inversely related to each other. More number of samples with hyposalivation had Candida colony formation that samples with normal salivation.

Similarly, salivation and number of Candida CFUs were inversely related to each other as more number of samples with hyposalivation was found to have ≥400 Candida CFU/ml than normal salivation samples.

DISCUSSION

Saliva is a clear, slightly acidic mucoserous exocrine secretion. Whole saliva is a complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, which contains oral bacteria and food debris.[6] It is composed of 99% of water and 1% of electrolytes, secretory proteins, immunoglobulins and organic molecules.[7]

Salivary function can be organized into five major categories: lubrication and protection, buffering action and clearance, maintenance of tooth integrity, antibacterial activity, taste and digestion.[7,8]

The average daily flow of whole saliva varies in health between 1 and 1.5 L. Salivary flow is categorized as unstimulated or resting and stimulated flow rate.[9]

Normal salivation: Unstimulated whole saliva is 0.1–1 ml/min, stimulated whole saliva is 0.5–3.5 ml/min.[9] Hyposalivation: Unstimulated whole saliva is <0.1 ml/min, stimulated whole saliva is <0.5 ml/min.[9] Hypersalivation: Unstimulated whole saliva is >1 ml/min, stimulated whole saliva is >3.5 ml/min.[9]

The saliva flow rate varies from person to person and is influenced by various factors such as degree of hydration, body position, exposure to light, previous stimulation, circadian rhythms and gland size.[10]

Xerostomia is a subjective complaint of dry mouth that may result from decrease in the production of saliva. Saliva plays a significant role in oral homeostasis. The presence of saliva usually is taken for granted, and it is not required for any life-sustaining functions. Nevertheless, its diminution or absence can cause significant morbidity and a reduction in a patient's perceptions of quality of life.[10]

When salivary function is diminished, there is more risk of patient's developing caries, experiencing denture

Table 1: Relationship between salivation and Candida colony formation

Candida colonies	Normal salivation, *n* (%)	Hyposalivation, *n* (%)	Total	χ^2	*P*
Colony formation	9 (35)	23 (96)	32	20.300	<0.001*
No colony formation	17 (65)	1 (4)	18		
Total	26 (100)	24 (100)	50		

*Significant association

Table 2: Relationship between salivation and number of Candida colony forming units formed

Colonies formation	Normal salivation, *n* (%)	Hyposalivation, *n* (%)	Total	χ^2	*P*
≥400 CFU/ml	1 (4)	19 (79)	20	29.501	<0.001*
Below 400 CFU/ml	25 (96)	5 (21)	30		
Total	26 (100)	24 (100)	50		

*Significant association

discomfort and having diseases such as candidiasis than there is in patients who have normal SFRs. Medications with antisialogogic effects are the most frequent causes of xerostomia. These include anticholinergic, antidepressant, antipsychotic, diuretic, antihypertensive, sedative and anxiolytic, antihistamine, opioid analgesic agents and nonsteroidal anti-inflammatory drugs.[11]

Some biological causes of xerostomia are having a history of radiation to the head and neck, diseases of the salivary gland, diabetes, alcoholic cirrhosis, cystic fibrosis, hormonal imbalance, autoimmune diseases (such as Sjögren's syndrome, rheumatoid arthritis, systemic lupus erythematosus) and other diseases. Social and psychological factors such as depression, anxiety and stress also are causes.[12]

In one of the similar studies by Torres *et al*.,[4] under same conditions, it was observed that 58% had hyposalivation, and another study by Torres *et al*.[4] showed that 22.1% had hyposalivation. Most of the patients in those studies who answered the questionnaire were patients from the University Hospital under treatment for some underlying disease. Torres *et al*. believed that the high prevalence of xerostomia in their study sample was attributed to underlying diseases and medication intake.[4]

The sample in our study had more females than males, 68% and 34%, respectively. More number of females (79%) had hyposalivation than males (21%) which were consistent with the other studies by Torres *et al*.[4] and Navazesh *et al*. who observed more number of females with hyposalivation than males. In those studies, they have mentioned that the volume of the submandibular salivary glands in females was 50% of that of the submandibular salivary glands of the males, and thus, they mentioned that this is the reason for the female predilection of the hyposalivation.[4]

Recent publications by Torres *et al*.[4] suggest that association between aging and hyposalivation may be related to other factors frequently present in older people, such as the presence of underlying diseases and their treatments.

Regarding hyposalivation, we did not observe any correlation between SFRs and age, a similar finding also reported by other authors. Although aging is associated with a loss of functional gland parenchyma, it can be clinically compensated for by the "reserve" capacity of the salivary glands, which is apparently high in healthy controls, as suggested by Vissink *et al*.[4] Although the feeling of xerostomia is commonly found in the elderly, this may also be explained by an impaired or altered psychosensory function affecting the perception of oral moisture or by changes in the chemical composition of the saliva.[4]

In the present study, out of 50 samples, 32 samples (64%) showed Candida colony formation, which was consistent with other study conducted by Torres *et al*.,[4] in which 67.9% of patients with xerostomia were colonized by Candida.

More number of samples with hyposalivation was found to have Candida colonization than samples with normal salivation which was similar to studies conducted by Torres *et al*.[4] and Navazesh *et al*.[5]

In a study conducted by Navazesh *et al*.,[5] who collected both unstimulated and stimulated whole saliva showed that unstimulated whole saliva was a better predictor for Candida counts and stimulated whole saliva was a good predictor. In the present study, stimulated whole saliva was used.

Various studies conducted by Torres *et al*.[4] showed more number of *C. albicans* species in saliva of xerostomia patients followed by *Candida parapsilosis* and *C. tropicalis* which was consistent with the present study.

In our study, out of fifty patients, 34 (68%) were medically compromised and were under one or many medications for the same. Among 34 medicated patients, 13 had normal salivation, 21 had hyposalivation. Among 16 nonmedicated patients, 13 had normal salivation and 3 had hyposalivation.

Of the 16 patients who had <400 CFU/ml, 12 patients had normal salivation and 4 had hyposalivation. While of the 18 patients with ≥400 CFU/ml, 17 patients had hyposalivation and 1 had normal salivation. This was similar to the study conducted by Torres *et al*.,[4] in which a significantly negative correlation was present between whole saliva flow rates and Candida counts in patients with medications.

Thus, the present study shows that there was a significant negative correlation between SFRs and Candida counts in patients with xerostomia, especially those who were under systemic medications.

CONCLUSION

In the present study to assess the relationship between the SFRs and Candida counts in patients with xerostomia, we have arrived at the following conclusion:

- Xerostomia was the most common subjective symptom in adult population, especially those who were on

medications for hypertension, diabetes, psychological disorder, thyroid dysfunction and cancer chemotherapy
- Xerostomia was associated with reduced SFRs, i.e., hyposalivation
- More number of females had hyposalivation than males
- Stimulated whole saliva can be a good predictor in assessing SFRs
- Statistically significant association was observed between reduced SFRs and increased Candida counts
- *C. albicans* was most common Candida species colonizing the mouth.

As the dental practitioners come across a number of geriatric and medically compromised patients, evaluation of whole saliva should be considered as part of routine clinical evaluation to identify patients at risk for candidiasis, prevent clinical implications and improve oral health-related quality of health of the patient. The method of whole saliva collection is simple, practical, inexpensive and a reliable method that can be easily used by all practitioners.

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Conflicts of interest
There are no conflicts of interest.

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