A prospective randomized trial to reduce oral Candida spp. colonization in patients with hyposalivation

Ensaiio clínico aleatório para reduzir a colonização oral de Candida spp. em pacientes com hipossalivação

Abstract: Low salivary flow rates are associated with higher oral Candida spp. counts, which may predispose to oral candidiasis. The aim of this study was to compare the effect of stimulating salivary flow rates with that of a regimen of chlorhexidine mouth rinse on the intensity of Candida colonization in patients with reduced salivary flow rates. Thirty-one outpatients were randomized to stimulate salivary output (group 1) or to receive chlorhexidine mouth rinses (group 2). Evaluations were performed at baseline (T₀), at end of treatment (T₁), and 15 days after last day of treatment (T₂). Chewing-stimulated whole saliva samples were collected at each visit. Group 1 showed a constant reduction in median cfu counts, although the difference was significant only between T₀ and T₂ (p = 0.004). Group 2 showed a reduction in median Candida cfu counts between T₀ and T₁ (p = 0.01), but the counts increased at T₂ (p = 0.01), and the difference between T₀ and T₂ was not significant (p = 0.8). In conclusion, patients who received saliva stimulation showed reductions of Candida cfu counts in saliva and a trend for increasing salivary flow rates between baseline and end of study evaluations. The use of chlorhexidine mouth rinses dramatically reduced Candida cfu counts, but when patients discontinued treatment, intensity of colonization rose again.

Descriptors: Saliva; Candida; Xerostomia; Homeostasis; Colony count, microbial.

Resumo: O fluxo salivar reduzido está associado a maior quantidade de Candida spp. na boca, predispondo a candidíase. O objetivo deste estudo foi comparar o efeito da estimulação salivar ao efeito do uso de bochechos de cloreoxidina sobre a intensidade de colonização por Candida em pacientes com fluxo salivar reduzido. Trinta e um pacientes de ambulatório foram aleatoriamente incluídos nos protocolos de estimulação salivar (grupo 1) ou de bochecho com cloreoxidina (grupo 2). As avaliações foram realizadas no dia inicial (T₀), ao final do tratamento (T₁) e 15 dias após o final do tratamento (T₂). A cada consulta foram coletadas amostras de saliva total estimulada. O grupo 1 mostrou uma redução constante nas contagens medianas de UFC de Candida, embora a diferença estatística tenha sido apenas entre T₀ e T₂ (p = 0.004). O grupo 2 mostrou redução nas contagens de UFC de Candida entre T₀ e T₁ (p = 0.01), mas a contagem de UFC aumentou em T₂ (p = 0.01), sendo a diferença entre T₀ e T₂ não significante (p = 0.8). Concluiu-se que os pacientes que realizaram procedimentos de estimulação salivar apresentaram uma quantidade de UFC de Candida salivar reduzida, além de apresentarem tendência ao aumento do fluxo. O uso de bochechos de cloreoxidina reduziu drasticamente a quantidade de UFC de Candida salivar, mas após o final do tratamento houve novo aumento.

Descritores: Saliva; Candida; Xerostomia; Homeostase; Contagem de colônia microbiana.
Introduction

Candida spp. are frequent colonizers of the oropharynx in humans, and high salivary Candida counts may predispose to oral candidiasis.6,28,29 It has been shown that low salivary flow rates (SFR) are associated with higher oral Candida counts.23,28,29 Therefore, increasing salivary output in subjects with low SFR could reduce oral Candida counts. Attempts to increase SFR include the use of sialogogue medications,13,27 as well as clinical procedures such as encouraging chewing14 and gustatory exposure.27 Other measures to reduce colonization by Candida include use of antimicrobial mouth rinses.12,20 In this study we evaluated the effect of stimulating SFR on the intensity of Candida colonization in patients with reduced SFR and high salivary Candida colony forming units (cfu) counts, and compared this strategy with a regimen of chlorhexidine mouth rinse, in a prospective randomized fashion.

Material and Methods

Patients’ population

This was a randomized trial in which two methods for reducing Candida spp. oral colonization were compared. Outpatients from the Dental School and from the University Hospital, Federal University of Rio de Janeiro (UFRJ), were randomly selected to answer a questionnaire about xerostomia.29 Patients who answered “yes” to at least one of the questions of the questionnaire were invited to participate in the study. Clinical and laboratory evaluations were performed, and patients who presented SFR < 1.0 ml/min25 and Candida spp. cfu counts ≥ 400 cfu/mL4 were included in the study. Exclusion criteria were: patients with oral candidiasis; patients with chewing-stimulated SFR ≥ 1.0 ml/min; patients with Candida cfu counts in saliva < 400 cfu/ml, and patients who received corticosteroids and antifungal agents. There were 124 patients evaluated, and 39 fulfilled the entry criteria. Twenty-three patients were randomized to group 1, and 16 patients to group 2. After randomization, 8 patients were excluded for the following reasons: in group 1, one patient started antifungal therapy and 4 patients dropped the study before second evaluation; in group 2, three patients dropped the study before second evaluation. Characteristics of the 31 evaluable patients (18 patients in group 1 and 13 patients in group 2) are shown in Table 1. All patients signed an informed consent. The study was approved by institutional ethical committee.
Study therapies
Patients were randomly assigned to one of two groups:
- Group 1 - Patients were instructed to stimulate salivary output during 15 days by drinking 2 L of water daily, chewing meals intensely, chewing sugarless gum\(^{13,14,27}\) (Trident\(^{\circledR}\), São Paulo, SP, Brazil) three times a day, and chewing ginger flakes\(^{27}\) (Ardrak\(^{\circledR}\), Hidrolândia, GO, Brazil) three times a day. Patients with a past history of gastritis were asked to use sugarless candies (Flópi\(^{\circledR}\), Lajeado, RS, Brazil) instead of gum,\(^{24}\) and those who were hypertensive received raw ginger root instead of the salted ginger flakes.\(^{11}\)
- Group 2 - Patients were given non-labeled 300 ml of a 0.12% chlorhexidine solution\(^{12,20}\) (Periogard\(^{\circledR}\), São Paulo, SP, Brazil), and were asked to rinse twice a day with 10 ml of the solution, during 1 minute, after 30 minutes of having performed oral hygiene procedures, after breakfast and supper, for 15 days.

Periogard\(^{\circledR}\), Trident\(^{\circledR}\) sugarless gum, and Ardrak\(^{\circledR}\) ginger flakes were obtained from the manufacturer.

Evaluation
Baseline evaluation consisted of medical history, clinical examination, sialometry and microbiological analysis. Samples of chewing-stimulated whole saliva were obtained under standard conditions.\(^{29}\) Saliva samples were collected between 9:00 AM and 11:00 AM, and no feeding, drinking, smoking or hygienic habits were allowed for 120 minutes prior to test section. Only the liquid component (not the foam) of saliva was measured. The SFR were determined as milliliters per minute. The samples of saliva were kept in a refrigerated recipient and taken to the Oral Microbiology Laboratory, UFRJ, within 2 hours.\(^{28}\) The samples where heated at 55°C for 2 minutes to disaggregate whole saliva components and facilitate microbial recovery, and were homogenized in a vortex (Supermixer\(^{\circledR}\), Melrose Park, IL, USA).\(^{28}\) A 0.1 ml sample of saliva was plated onto CHROMagar Candida\(^{\circledR}\) (Paris, France) and incubated at 37°C for 72 hours. Total and colony-color-specific cfu were counted. One representative cfu of Candida of each color was isolated and Candida alb-...
p = 0.01), but at T2, there was an increase in median cfu counts (Δ2 p = 0.01), and the difference in median cfu counts at baseline (T0) and end of study (T2) was not statistically significant (Δ3 p = 0.8).

Differences in SFR were also measured at each period of collection, for each group. Graph 2 shows SFR at the three periods of collection. In group 1, no statistical differences were seen in median SFR from T0 to T1 (Δ1 p = 0.15) and from T1 to T2 (Δ2 p = 0.72), but there was a trend for increasing SFR between the baseline and end of study evaluations (Δ3 p = 0.07). In group 2, median SFR were higher from T0 to T1 (Δ1 p = 0.33) and reduced from T1 to T2 (Δ2 p = 0.40). Comparing T0 to T2, there was a trend for higher SFR at end of study in this group (Δ3: p = 0.07).

We analyzed the counts of most frequent Candida species (Table 2). In group 1, there were no statistically significant differences in cfu counts of C. albicans at the three periods, whereas for C. parapsilosis, there was a trend for increasing the intensity at end of study comparing to baseline. In group 2, there was a significant reduction in C. albicans counts at T1 comparing to T0, but like total cfu counts, it rose again at end of study. Regarding C. parapsilosis, there was no significant difference comparing the three evaluations.

**Discussion**

This study showed that the use of chlorhexidine mouth rinses dramatically reduced Candida cfu counts, but after stopping the rinses, there was an increase in cfu counts, and comparing baseline and end of study cfu counts, the difference was not statistically significant. On the other hand, salivary stimulation (group 1) resulted in a constant reduction in Candida cfu counts, although less intense than in group 2 (Graph 1).
Regarding SFR, patients who were instructed to stimulate salivary output had an increase in SFR (p = 0.07) comparing baseline and end of study evaluations. Surprisingly, patients assigned to receive chlorhexidine mouth rinses also had an increase in SFR (p = 0.07) comparing baseline and end of study evaluations. Therefore, the significant and long-lasting reduction in Candida cfu counts observed in group 1 may be explained by an increase in salivary flow rates.

Xerostomia has been reported as a side effect of chlorhexidine use, but no study evaluated the effect of chlorhexidine on SFR. We don’t have a clear explanation for the increase in SFR observed at end of study in group 2, but we suppose that these patients may have changed their habits, incorporating practices that increase SFR, such as chewing gums or candies, drinking more water, even if they were not instructed to do so. Unfortunately we did not evaluate this possible bias.

Another interesting observation of the present study is the increase in SFR that occurred after discontinuation of salivary stimulation in group 1 patients. A possible explanation for this result is the possibility that once salivary glands are stimulated, the output continues to increase even after ceasing the stimulus. Indeed, some studies have shown a long-term effect of gum-chewing in SFR.

Stimulating the output of SFR seems to enhance oral homeostasis, and thus promote natural protection. Continuous salivary flow protects by its cleansing effect and by the antimicrobial action of salivary proteins. Many salivary proteins have activity against Candida. Histatin is a peptide that shows potent candidacidal effect. Moreover, secretory IgA inhibits Candida adherence to oral mucosa. It has been demonstrated that chewing increases the secretion of IgA, as well as other salivary proteins. Therefore, it is possible that patients who stimulated salivary output had an increase in salivary IgA and proteins, and this exerted protection against Candida. Further studies evaluating sialochemistry should be carried out in order to support our hypothesis.

Ginger (Zingiber officinale), one of the gustatory stimulants of group 1, is used mainly for oral and gastric disturbances. Some studies have investigated antimicrobial in vitro activities of ginger, but there has been no clinical trial conducted to investigate its antimicrobial and salivary stimulant properties.

Candida susceptibility to chlorhexidine has been evaluated in recent studies. In denture plaque biofilms, Candida has shown better response to chlorhexidine than to fluconazole and miconazole. However, clinical studies using chlorhexidine for prophylaxis of oral candidiasis in chemotherapy or radiotherapy patients have shown conflicting results. Resistance to chlorhexidine has been reported in some phenotypic resistant subpopulations of C. albicans. In the present study, the effect of chlorhexidine was evaluated in the two most frequent species of Candida, but the small number of patients hampers any conclusion regarding this issue.

These results may have important clinical and experimental implications. First, health care workers may apply these measures in order to stimulate salivary output and to reduce Candida colonization. Furthermore, clinical and laboratory research must be performed to study the effects of salivary stimulation on sialochemistry.

Conclusion

Patients with reduced salivary flow rates and high Candida cfu in saliva that received salivary stimulation showed reduction of Candida cfu counts in saliva and a trend for increasing SFR between baseline and end of study evaluations. The use of chlorhexidine mouth rinses dramatically reduced Candida cfu counts, but when patients finished treatment, the intensity of colonization rose again.

Acknowledgements

We wish to thank the staff personnel of the University Hospital and Dental School, Federal University of Rio de Janeiro (UFRJ), for referring patients; the Dental School students, who applied the questionnaires; and Fernando A. C. Magalhães, for the laboratorial support.
References


