Original Article

Evaluation of the Amount and pH of Saliva of Edentulous Patients before and after Using Probiotics.

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Abstract:

Introduction: Elderly individuals, with or without full dentures, have often been offered probiotics to aid with digestion. An increase in saliva production due to the use of probiotics is noted. Therefore, it was decided to conduct this research to determine the effects of probiotics on the quantity and pH of saliva in patients who are entirely denture-free after 60 days of use.

Aim: The goal of this study is to evaluate whether or not probiotic supplementation causes an increase in saliva production in edentulous individuals.

Methods: Patients with missing teeth were used in the research. The pH was measured after collecting baseline amounts and baseline amounts of both unstimulated and stimulated saliva. Then, once a day for 60 days, patients took capsules containing probiotics at 3.3 107 CFU/g. Patients' saliva was collected, and its volume and pH were determined for comparison to the initial measurements.

Results: After 60 days of taking probiotics, the average amount of saliva, both unstimulated and stimulated, is higher than it was at the beginning of the study. P <0.001 indicates a statistically significant difference. After 60 days, the mean pH was somewhat higher than it had been at the start (0.010).

Conclusion: Those with xerostomia or hyposalivation may benefit from taking probiotics, as this study found that they increased saliva production in patients who were completely edentulous.

Keywords: Edentulous patients, hyposalivation, probiotics, saliva, xerostomia

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Introduction:

"Live microorganisms that confer health benefits on the host when administered in enough amounts constitute probiotics. This strategy has been successfully used to the management of intestinal problems, and its mechanism of action seems to include colonization resistance and/or modulation of the immune system [1,2]. Oral problems such as dental caries and periodontal diseases have been linked to variations in the microbial makeup and activity of a biofilm, as well as the host's sensitivity to this shift [3, 4].Probiotics are now being studied as a means to prevent or cure these conditions. Products of many types have long made use of probiotics (e.g., tablets, powder, tooth, milk, and salt). It is hypothesized that probiotics' therapeutic

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effects on intraoral bacteria occur through a process similar to that which occurs in the intestines, where microorganisms interact with one another at both the systemic and local levels. The results of a literature study on probiotic bacteria as a possible anti-caries strategy could not rule out the possibility that these bacteria may disrupt dental biofilm. Salivary mutans streptococci levels were observed to be decreased by taking probiotics for shorter periods of time in a number of systematic evaluations on probiotics usage in caries prevention. Evidence from a meta-analysis and comprehensive review suggests that dairy probiotics can lower levels of streptococcus mutans, raise saliva pH, and increase plaque index.

The majority of probiotics research has focused on strains from the Lactobacillus, Streptococcus, and Bifidobacterium genera. Biofilm development on vocal prostheses can be prevented and treated [4]. The secondary results of the research show that probiotics help those who have problems with hyposalivation. Saliva is an intricate fluid composed of electrolytes, glycoproteins, and enzymes that aid in digestion, give us a sense of taste, and keep the mucosa clean and healthy. Patients who are entirely edentulous have an even greater need for sufficient saliva.

When taken in sufficient doses, some types of living microorganisms can improve a person's health, as defined by the World Health Organization (WHO). Russian Nobel laureate Elie Metchnikoff was the first to suggest that eating specific microbes may be good for the digestive system. Many research since then have demonstrated that probiotics are useful for preventing and treating gastrointestinal, allergy, and respiratory illnesses. Miller et al. [4] conducted an early investigation exploring the potential of different bacteria in dentistry. In the future, research showed that taking probiotics led to a decline in oral infections. Many studies have indicated a reduction in the numbers of Streptococcus mutans and Candida albicans. Ideally, the oral microbiota will diversify, with commensals taking the lead. Antimicrobial compounds secreted by probiotic bacteria are thought to influence immunological systems and to compete with cariogenic microbes for adhesion sites or dietary substrates. It follows that the use of probiotics can alter the make-up of one's oral microbiome, leading not just to a decrease in certain infections. There must be cariogenic microbes, nutritional substrates (high-carbohydrate diets), and enough time for the bacterium to make contact with sensitive tissues in order for caries to occur. As an added benefit, probiotics can lessen the likelihood of caries by boosting saliva production and therefore decreasing the amount of time that bacteria spend clinging to teeth. Scientists have also shown that probiotic supplementation can elevate salivary levels of secretory immunoglobulin A (sIgA). Inhibiting microbial adhesion, protecting the host against antigen absorption from mucosal surfaces, reducing inflammation, boosting phagocytosis, and neutralizing microbial toxins and invasive infections are all functions of immunoglobulins. Salivary immunoglobulins, including secretory immunoglobulin A, have been shown to have a significant role in reducing the risk of dental caries. Plaque development may be slowed by probiotics because of changes to the oral microbiota, increased salivary flow, and increased sIgA secretion [5, 6].

Saliva also has a substantial effect in reducing stomatitis of prosthetic etiology, a condition experienced by a sizable percentage of people who use total or partial dentures [5]. Probiotics have been shown to effectively reduce the risk of xerostomia, and this has been recognized and remarked upon as well. However, no studies have been conducted to validate these results in edentulous patients as of yet.

Material and Method: In order to conduct this interventional prospective study, patients who were missing all of their teeth were enlisted from the prosthodontics clinic. Patients were enrolled if they were entirely edentulous, 60 years or older, free of chronic debilitating disease or medication, oral pathology-free, and had full comprehension and consent to take part in the study. Over the course of two months, forty patients (30 men and 10 females) were analyzed. Those who did not give informed consent or who refused to participate were left out of the study.

Probiotics capsules, a saliva collection kit with a beaker, gauze, a scale, and a pH meter were the instruments and materials employed. Lactobacillus acidophilus, Lactobacillus rhamnosus GGHS111, and Bifidobacterium bifidum were the probiotic strains used in the research. "These strains will be combined in powder form to yield a potency of 108 CFU (3.3 107 CFU of each) per capsule, which will then be administered to the patient."

The institution had previously granted its prior ethical approval. Before they completed the written informed permission form, participants received information about the study's objectives and research procedures. At the beginning of the study, the participants' oral and dental health was evaluated. A comprehensive history was also gathered. The saliva was collected using two techniques:

1. The patient was asked to spit into a pre-weighed beaker any saliva that had collected on the bottom of their mouth without chewing or speaking. The duration of this exam was a swift five minutes. Hyposalivation was defined as a basal salivary flow rate of less than 0.1 ml/min in the absence of stimulation.
2. After five minutes of being told to chew on a piece of preweighed gauze, the patient was told to spit the wet piece of gauze and the collected saliva into a preweighed beaker. This was done to determine how much saliva had built up. It was determined that hyposalivation was present at a stimulated flow rate of 0.8 ml/min.

The method for gathering saliva that is often advised is this one. Two further methods for collecting saliva are the SalivaBio Oral Swab and passive drool method.

The pH of the saliva was measured using a pH meter after the saliva sample was collected. The next step was to give each patient 60 probiotic pills, which they were directed to take every day between eight and ten in the morning with water for 60 days. The patients' daily probiotic capsule intake was observed over those 60 days by routine follow-ups. Once the interventional phase had lasted 60 days, the patients were called back. All of the test takers were asked to abstain from eating and drinking for the preceding hour. Water was the only liquid they were allowed to consume.

Statistic Evaluation:

Using descriptive analysis, we determined the average and the standard deviation. The statistical significance of mean differences was assessed using the paired t-test. Every piece of data was shown as a mean SD. The cut-off for statistical significance was set at 0.05.

Result:

For this study, 40 participants were selected at random. Table 1 displays the total number of patients and the breakdown by age group. Patients between the ages of 65 and 65 made up the biggest demographic (n=22), followed by those between the ages of 66 and 70 (n=7), 71 and 75 (n=5), and 76 and 80 (n=5). Fewest patients (n=1) were in the oldest age group (81–85 years). (Table 1)

Table 1: Number of patients in each age group

Age groups (years)	Number of patient
60-65	22
66-70	7
71-75	5
76-80	5
81-85	1

Table 2: Describes the frequency distribution before and after 60 days for the whole stimulated, unstimulated saliva and pH.

Table 2: Frequency Distribution

	Unstimulated Saliva	Unstimulated Saliva	Stimulated Saliva	Stimulated Saliva	pH	pH
	0 week	60 days	0 week	60 days	0 week	60 days
Mean	0.945	1.257	2.045	2.490	7.910	7.955

*SD	0.659	0.725	0.645	0.702	0.245	0.195
Median	0.75	1.30	2.345	2.490	7.900	7.800
*IQR	0.65	0.947	0.895	1.125	0.100	0.000

*SD: Standard deviation, *IQR: Interquartile Range

According to the paired t-test statistics, after 60 days, the mean unstimulated and stimulated saliva from baseline increased. P0.001 indicates a statistically significant difference. There was reportedly a 0.009 point shift in mean pH between the first reading and the 60-day mark. (Table 3)

Table 3: Paired t-Test: (Unstimulated saliva, stimulated saliva and pH).

	Mean difference	Standard deviation	Standard Error Mean	P-value
Unstimulated saliva	-0.425	0.250	0.050	<0.001
Stimulated saliva	-0.445	0.165	0.035	<0.001
pH	-0.009	0.098	0.025	0.692

Discussion:

The purpose of this research was to examine how probiotics could influence saliva production in people who are toothless. So, we chose 40 edentulous individuals and had their saliva tested for volume and pH. Then, over the subsequent 60 days, participants were instructed to take three separate capsules containing the probiotic strains "Lactobacillus rhamnosus GG-HS111, Lactobacillus acidophilus HS101, and Bifidobacterium bifidum." Probiotic pill use was a topic of ongoing conversation with the patients. Following the collection of saliva samples and the taking of relevant measures, patients were contacted to schedule a follow-up appointment for the intervention. The sample readings underwent analysis by being compared to the reference values.

Three probiotic strains are capable of influencing salivation, according to the study. Saliva production was found to have greatly risen compared to baseline, despite the fact that there was no detectable change in pH.

The research's results are in line with Hataka's study's secondary findings [7], which were also obtained from this study. The primary purpose of the research was to determine whether the use of probiotics might lower the incidence of oral candida in the elderly. Through the course of the study, researchers discovered that a decrease in oral Candida was correlated with an increase in saliva production. There is no relevant literature since no previous studies have looked at the impact of probiotics on saliva production.

The researchers Sutula J. et al. examined the levels of volatile sulfur compounds (VSCs) in morning breath, spit microbiota, and the dorsal tongue lining of solid dentate people (n = 22) over the course of seven weeks. The review included three parts: a baseline visit to obtain "control" levels of oral boundaries before using the probiotic item; a four-week time of daily use of one 65-ml container of Yakult; and a fourteen-day waste of time period. Each bottle of Yakult contains roughly 6.510 practical cells of Lactobacillus casei strain Shirota (LcS). A wide array of specialized media were used to determine the diversity and viability of the microorganisms in the oral cavity. We tested if LcS was present in the mouth using a unique, specialized medium dubbed "LcS Select." The amounts of volatile sulfur compounds (VSCs) in first thing in the morning breath were measured using the portable sulphur monitors Halimeter(®) and OralChroma (TM).During the probiotic intervention period, healthy dentate people (n = 19) with saliva and tongue plaque samples demonstrated a substantial, albeit transitory, and consumption-dependent presence of LcS. LcS was no longer observable with culture after two weeks without consumption. With the exception of a few outliers when Halimeter readings were considerably lowered throughout the probiotic intervention period, "morning breath scores measured with Halimeter and OralChroma were not significantly impacted throughout the investigation. Normal changes in the populations of the regional acidogenic species had little effect on the abundance of Candida and anaerobic species, including odoriferous Gram-

negative anaerobes." In spite of the fact that healthy dentate people who ate Yakult did not experience any appreciable ecological changes in their mouths, they came to the conclusion that the study's findings did provide evidence for LcS's transitory and intake-dependent existence. Future studies might concentrate on those who are more prone to oral infections because use of this probiotic may have a considerable effect on those with clinically confirmed halitosis or an ill-defined microbiota (such as a greater presence of peri pathogens)[8,9].

The short-term efficacy of probiotics in reducing oral Candida infection in elderly denture wearers with asymptomatic candidiasis was studied by Ishikawa KH et al. A double-blind, randomized experiment with two groups—probiotic and placebo—was conducted on 59 denture wearers who had oral Candida spp. but no clinical symptoms. All patients received instructions on how to daily care for their dentures. All of the patients' palate mucosas initially had Candida. A total of 55 people finished the study. After the trial period, the probiotic group's detection rate of Candida spp. was 16.7% whereas the placebo group's rate was 92.0%. The quantity of Candida infection, species that colonized the area, the age of the dentures, and other preexisting characteristics had no impact on the probiotic regimen's ability to lessen symptoms. "They came to the conclusion that the probiotic treatment was effective in reducing Candida colonization of the oral cavity in senior denture wearers who did not have signs of oral candidiasis [10]. As a result, it appears that oral candidiasis may be prevented with this multispecies probiotic.

Denture wearers are particularly susceptible to oral Candida infection, thus TY Miyazima et al. looked at the effects of two experimental cheeses containing probiotics on this common problem. For eight weeks, 60 denture wearers with oral Candida were fed either cheese treated with Lactobacillus acidophilus NCFM (T1) or Lactobacillus rhamnosus Lr-32 (T2). For comparison, group C received no cheese. Initially, at the beginning of the experiment, and again at week eight, mouthwash samples were obtained to determine the number of Candida (CFU/mL). Each group's mean Candida spp. (log CFU/mL) baseline levels were comparable. Despite not being in group C, groups T1 and T2 saw a considerable decline in the mean Candida levels. No matter the kind of Candida that infested the participants' mouths, their ages, or whether they wore bi- or unimaxillary dentures, the levels of Candida in the mouth dropped. Daily cheese consumption was established [11].

Although the exact reason for this increase is unknown, a study has suggested that probiotics may somehow alter the chemical composition of saliva, including mucin and salivary immunoglobulin concentrations, as has been shown in animal [12] and in vivo studies [13], which would then affect the type and amount of saliva secreted. It has been discovered that probiotics can increase the synthesis of hormones. Consuming probiotics has also been shown to boost an animal's milk production [14,15]. This shows that probiotics do have an effect on how much fluid the body produces. Probiotics have been shown to boost parotid gland production of salivary beta adhesion 2 [16]. The conclusion that probiotics may alter the epithelial cells of salivary glands like the parotid to increase saliva production seems reasonable. More study is needed to pin down the precise mechanism of action.

Salivary flow rate is yet another essential factor for maintaining dental and general health. The immune-modulating effects of probiotics may be beneficial to all species or strain-specific for some. No published investigations that were similarly structured exist as far as we are aware [16]. According to published research, fluctuations in salivary flow rates, or sIgA levels, may also have an impact on salivary protein concentrations. In xerostomia patients, such as pregnant women and students under exam-related stress, a rise in salivary sIgA has been noted, for instance.

One key goal of these interventional trials is to improve adherence to the daily probiotic dosage that is advised for home use. In this study, an effort was made to persuade participants to take probiotics on a regular basis by contacting them. The duration of this study was only two months, as well. So, it is possible to plan a comparable study that involves a larger sample size.The conclusion that probiotics promote salivation in people with edentulous teeth can be reached as a result of the null hypothesis being refuted. The study's statistically significant results showed that probiotics can help individuals with

xerostomia and hyposalivation as well as those who are entirely edentulous in terms of salivation. Although there is emerging evidence that probiotics have beneficial benefits in the mouth, further investigation is required to pinpoint the particular molecular pathways involved.

Conclusion:

Within the constraints of the study, it can be said that probiotics can boost salivation in individuals who are entirely denuded, and can thus be used sparingly in those who have xerostomia or hyposalivation.

References:

1. Turner M, Jahangiri L, Ship JA. Hyposalivation, xerostomia and the complete denture: A systematic review. J Am Dent Assoc 2008;139:146-50.
2. Rohim RA, Ahmad WMAW, Ismail NH, Yaqoob MA, Alam MK, Ghazali FMM. Study of oral lactobacillus towards developing a comprehensive structured for integrated exponential regression model. Bangladesh J Med Sci. 2020 Mar;19(3):552-7.
3. 3.Niedermeier WH, Krämer R. Saliva and dentures. J Prosthet Dent 1992;67:211-6
4. Miller, C.; Kleinman, J. Effect of Microbial Interactions on in Vitro Plaque Formation by Streptococcus Mutans. J. Dent. Res. 1974, 53, 427–434.
5. Hamzan NI, Fauzi FH, Taib H, Mohamad S. Simple andrapid detection of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans by loop-mediated isothermal amplification assay. Bangladesh J Med Sci. 2018 Jun 29;17(3):402-10.
6. Miyazima TY, Ishikawa KH, Mayer M, Saad S, Nakamae A. Cheese supplemented with probiotics reduced the Candida levels in denture wearers-RCT.Oral Dis. 2017 10.1111/odi.12669. Epub 2017 May 16. Oct;23(7):919-925. doi:
7. Hatakka K, Ahola AJ, Yli-Knuuttila H, Richardson M, Poussa T, Meurman JH, et al. Probiotics reduce the prevalence of oral candida in the elderly – A randomized controlled trial. J Dent Res 2007;86:125-30.
8. 8.Sanghvi U, Chhabra T, Sethuraman R. Effect of probiotics on the amount and pH of saliva in edentulous patients: A Prospective study.J Indian Prosthodont Soc. 2018 Jul-Sep;18(3):277-281. doi: 10.4103/jips.jips_121_18.
9. Sutula J, Coulthwaite LA, Thomas LV, Verran J. The effect of a commercial probiotic drink containing Lactobacillus casei strain Shirota on oral health in healthy dentate people.MicrobEcol Health Dis. 2013 Oct 29;24. doi: 10.3402/mehd.v24i0.21003. eCollection 2013.
10. 10.Ishikawa KH, Mayer MP , Miyazima TY , Matsubara VH, Silva EG, Paula CR, Campos TT, Nakamae AE. A multispecies probiotic reduces oral Candida colonization in denture wearers.J Prosthodont. 2015 Apr;24(3):194-9. doi: 10.1111/jopr.12198. Epub 2014 Aug 20.
11. Miyazima TY, Ishikawa KH, Mayer M, Saad S, Nakamae A. Cheese supplemented with probiotics reduced the Candida levels in denture wearers-RCT.Oral Dis. 2017 10.1111/odi.12669. Epub 2017 May 16. Oct;23(7):919-925. doi:
12. Negretti F, Casetta P, Clerici-Bagozzi D, Marini A. Researches on the intestinal and systemic immune-responses after oral treatments with Lactobacillus GG in rabbit. Dev Phisiopath Clin 1997;7:15-21.
13. Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA. Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. Gut 2003;52:827-33.
14. Kritas SK, Govaris A, Christodoulopoulos G, Burriel AR. Effect of bacillus licheniformis and Bacillus subtilis supplementation of ewe's feed on sheep milk production and young lamb mortality. J Vet Med A Physiol Pathol Clin Med 2006;53:170-3.
15. Nawaz A, Batool H, Kashif M, Abbas A, Shahzad F, Tahir R, Afzal N. Immune profiling of saliva in patients with and without dental caries. Bangladesh J Med Sci. 2019 May 30;18(3):536-9.
16. Kusumaningsih T, Subijanto MS, Indrawati R, Devijanti RR. The level of beta defensin-2 in saliva and its expression in parotid gland epithelial cells after probiotic (Lactobacillus reuteri) induction to inhibit Streptococcus mutans in caries. Eur J Dent 2016;10:556-60.