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Comparison of Antibacterial Effect of Probiotic Yogurt and Xylitol-Containing Chewing Gum in Geriatric Patients: A Randomized Controlled Clinical Trial

Usporedba antibakterijskoga učinka probiotičkog jogurta i žvakaće gume koja sadržava ksilitol kod gerijatrijskih pacijenata: randomizirano kontrolirano kliničko istraživanje

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Abstract

Objectives: To evaluate the effect of probiotic bacteria in yogurt on *Streptococcus mutans* (MS) count, plaque adherence and salivary pH compared to xylitol-containing chewing gum in geriatric patients. **Material and methods:** Total number of 96 high caries risk geriatric patients were randomized into two equal groups (n=48). Group 1 (intervention group) received probiotic yogurt (Activia, Danone) once per day, and group 2 (control group) received xylitol chewing gum (Trident original) three times per day. The primary outcome was salivary *Streptococcus mutans* count and secondary outcomes were interdental plaque *Streptococcus mutans* count, salivary pH and bacterial adherence. **Results:** For *Streptococcus mutans* count in saliva and plaque, a statistically significant reduction in the level of MS over all the examined follow up periods of the study in probiotic yogurt group as well as xylitol gum group was found. An intergroup comparison for salivary MS count showed statistically significant difference between the two materials in a two week and a three month period of time and there was no statistically significant difference between both materials at one month time period. Salivary pH results showed statistically significant increase in pH in both groups along the follow-up periods. Bacterial adherence results showed statistically significant reduction in both groups. **Conclusions:** Probiotic yogurt is an effective antibacterial agent against salivary and plaque bacteria in geriatric patients.

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Introduction:

Dysbiosis of the biofilm, with change of the bacterial composition, occurs with stress factors (1). Many factors can be classified as caries predictors: dietary changes, neglecting of oral hygiene, medical treatment or medicines that affect salivary flow and changes in host immune response are considered risk factors (2). These factors lead to flourishing of cariogenic *Streptococcus mutans*, the most important bacterial species involved in the demineralization of tooth tissue and initiation of dental caries (3).

In geriatric population, people's immune functions decline, the lack of awareness increases, and their ability for oral hygiene care decreases (4). Antibacterial compounds such as xylitol have been suggested to reduce the activity of cariogenic bacteria (2). The delivery of xylitol via chewing gum is considered as a convenient, effective and simple way for geriatric patients (5). However, long term use of xylitol containing products causes adverse effects such as cramps, bloating, constipation, flatulence and loose stool or diarrhea (6).

Uvod

Disbioza biofilma s promjenom bakterijskoga sastava pojavljuje se uz čimbenike stresa (1). Mnogi od njih mogu se klasificirati kao prediktori karijesa. Promjene u prehrani, zanemarivanje oralne higijene, liječenje ili lijekovi koji utječu na protok sline i promjene u imunosnom odgovoru domaćina smatraju se čimbenicima rizika (2). Svi oni potiču povećanje broja kariogenih bakterija *Streptococcus mutans* (*S. mutans*), najvažnije bakterijske vrste uključene u demineralizaciju zubnoga tkiva i inicijaciju zubnoga karijesa (3).

U gerijatrijskoj populaciji opada imunost pojedinaca, smanjuje se svijest i sposobnost za održavanje oralne higijene (4). Smatra se da antibakterijski spojevi poput ksilitola smanjuju aktivnost kariogenih bakterija (2). Izlaganje ksilitolu putem žvakaće gume smatra se prikladnim, učinkovitim i jednostavnim načinom za gerijatrijske pacijente (5). No dugotrajna uporaba proizvoda koji sadržavaju ksilitol uzrokuje štetne učinke kao što su grčevi, nadutost, zatvor i rijetka stolica ili proljev (6).

Probiotics are live microorganisms that provide benefits to oral health. They adhere to the oral mucosa and teeth surfaces and become part of the biofilm preventing the adhesion, colonization, and proliferation of other cariogenic bacteria inhibiting the formation of pathogenic plaque (7)

Probiotics are found in different dairy products, including yoghurt, which are known as carriers of probiotics (3). Therefore, the incorporation of dairy products that do not require a caregiver in their daily routine, can be used as a simple means of protection from caries in geriatric patients.

It was found beneficial to test the null hypothesis that probiotic yogurt has similar antibacterial effect in plaque and saliva, similar pH and similar effect on bacterial adherence as xylitol-containing chewing gum in geriatric patients.

Material and methods

Study setting

This randomized controlled clinical study was conducted at the Faculty of Dentistry, Cairo University, Egypt. The protocol of this study was registered in www.clinicaltrials.gov/ database, with unique identification number NCT03889015. All the procedures done in this study involve human participants, and the study has been approved by the Research Ethics Committee (CREC) of Faculty of Dentistry, Cairo University, and approval number 19-4-15.

This study was conducted for approximately 3 months and it was single blinded, parallel-arm, randomized clinical trial with 1:1 allocation ratio. The participants of the study were randomly assigned into two equal groups.

Sample size calculation

The sample size was calculated based on the previous study by Hanno et al. in 2011 in which the probability of patients without reduction in bacterial count ($\geq 10^5$) after 3 months for xylitol chewing gum was (0.471). The estimated probability of patients without reduction in bacterial count ($\geq 10^5$) after 3 months for probiotic yogurt was (0.171) with a difference of 30%. By adopting an alpha (α) level of 0.05 (5%), power (80%), the predicted sample size (n) was a total of 74 (37 patients per group) to be able to reject the null hypothesis that the success rates for case and controls are equal. The sample size was increased by (30%) to compensate for possible dropouts during follow-up to be total of (96) cases i.e. (48) for each group. The sample size calculation was performed using G*Power 3.1.9.2, Universität Kiel, Germany.

Eligibility criteria

Subjects were assessed for eligibility to be included in the study. High caries risk geriatric patients (over 65 years old) with *Streptococcus mutans* count more than 10^5 CFU (by culturing on *mitis salivarius* bacitracin agar) were included in the study. Their cognitive ability had to be sufficient to understand consent procedures.

Subjects who were milk intolerant or habitual consumers of xylitol were excluded from the study. The geriatric patients who used antibacterial mouthwash or were under any antibiotic treatment were not included in the study.

Probiotici su živi mikroorganizmi koji pridonose zdravlju usne šupljine. Prianjaju na sluznicu usne šupljine i površine zuba te postaju dio biofilma i tako sprječavaju adheziju, kolonizaciju i proliferaciju drugih kariogenih bakterija inhibirajući stvaranje patogenoga plaka (7).

Probiotici se nalaze u različitim mliječnim proizvodima, uključujući jogurte koji su poznati kao nositelji probiotika (3). Zato se uključivanje mliječnih proizvoda u svakodnevnu rutinu, jer ne zahtijevaju angažman njegovatelja, može kod gerijatrijskih pacijenata primijeniti kao jednostavno sredstvo zaštite od karijesa.

Cilj je bio testirati nultu hipotezu da probiotički jogurt kod gerijatrijskih pacijenata postiže sličan antibakterijski učinak na plak i slinu, sličan pH i sličan učinak na adherenciju bakterija kao žvakaća guma koja sadržava ksilitol.

Materijali i metode

Studijski dizajn

Ovo randomizirano kontrolirano kliničko istraživanje provedeno je na Stomatološkom fakultetu Sveučilišta u Kairu, Egipat. Njegov protokol registriran je u bazi podataka www.clinicaltrials.gov s jedinstvenim identifikacijskim brojem NCT03889015. Svi postupci primijenjeni u ovom istraživanju, a uključuju ljudske sudionike, bili su usklađeni s etičkim standardima Odbora za etiku u istraživanjima Stomatološkog fakulteta Sveučilišta u Kairu – broj odobrenja 19-4-15.

Ovo je jednostruko slijepo, paralelno, randomizirano kliničko istraživanje s omjerom raspodjele 1 : 1 i trajalo je tri mjeseca. Sudionici su nasumično raspoređeni u dvije jednake skupine.

Izračun veličine uzorka

Veličina uzorka izračunata je na temelju ranijeg istraživanja Hannoa i suradnika iz 2011. u kojemu je vjerojatnost pacijenata, bez smanjenja broja bakterija ($\geq 10^5$), poslije tri mjeseca za žvakaću gumu sa ksilitolom bila 0,471. Procijenjena vjerojatnost pacijenata bez smanjenja broja bakterija ($\geq 10^5$) poslije tri mjeseca za probiotski jogurt iznosila je 0,171, s razlikom od 30 %. Prihvaćanjem alfa (α) razine od 0,05 (5 %) snage (80 %), predviđena veličina uzorka (n) bila je ukupno 74 (37 pacijenata u skupini) kako bi se mogla odbaciti nulta hipoteza da su stope uspjeha za slučajeve i kontrole jednake. Veličina uzorka povećana je za 30 % da bi se kompenzirala moguća odustajanja tijekom praćenja i iznosila je ukupno 96 slučajeva, tj. 48 za svaku skupinu. Izračun veličine uzorka izveden je s pomoću G*Powera 3.1.9.2 (Universität Kiel, Njemačka).

Kriteriji uključivanja

Procijenjeno je ispunjavaju li ispitanici uvjete za sudjelovanje u istraživanju. Uključeni su gerijatrijski pacijenti s visokim rizikom od karijesa (iznad 65 godina) s razinom bakterija *Streptococcus mutans* iznad 10^5 CFU-a (s kultivacijom na agaru *mitis salivarius bacitracin*). Njihovo kognitivno svojstvo moralo je biti dovoljno za razumijevanje postupaka i davanje pristanka.

Ispitanici koji su bili netolerantni na mlijeko ili uobičajeni konzumenti ksilitola bili su isključeni iz istraživanja. Gerijatrijski pacijenti koji su se koristili antibakterijskom vo-

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Participants were recruited in the study to fulfil the eligibility criteria and screening continued until the target geriatric population (96 participants) was achieved. An Arabic informed consent supplied by the Research Ethics Committee (REC) at Faculty of Dentistry, Cairo University containing all the ethical aspects of the trial was signed by the eligible participants prior to the initiation of the study.

Randomization, Sequence generation and blinding

Simple randomization was done by generating numbers from 1:96 using Random Sequence Generator, Randomness and Integrity Services Ltd (www.randomization.com). Each generated random number represented assigning intervention and comparator in a random manner. The investigator and the microbiologist were blinded to the material assigned. However, the patients could not be blinded due to the difference between probiotic yogurt and chewing gum.

The materials used were Probiotic yogurt (Activia; Danone, Egypt) and xylitol Chewing Gum (Trident Original) presented in Table 1. For Intervention Group, participants had to eat 100 grams of the provided probiotic yogurt 10 minutes after dinner. They were advised not to brush their teeth until one-hour later[3]. For Comparator Group, the participants were instructed to chew the provided gum three times daily for five minutes after each meal (3).

For both groups, the participants were instructed not to take other probiotic products or mouth rinse throughout the study period. All participants were asked to continue their routine toothbrushing and flossing habits during the study period.

Salivary and interdental plaque MS counts, salivary pH and bacterial adherence were evaluated at different assessment times T0: before taking either the intervention or the comparator, at T1: two weeks, at T2: one month and T3: three months.

For salivary *Streptococcus mutans* count, the subjects were instructed not to eat or drink anything for at least one hour before the collection of saliva sample on the day of saliva collection. The participants were asked to rinse their mouth with

dicom za ispiranje usta ili su se liječili nekim antibiotikom također nisu uključeni u istraživanje.

Svi koji su ispunjavali kriterije uključeni su u istraživanje, a probir je nastavljen sve dok nije postignuta ciljana gerijatrijska populacija od 96 sudionika. Informirani pristanak na arapskome jeziku koji je odobrio Odbor za etiku u istraživanjima Stomatološkog fakulteta Sveučilišta u Kairu, a sadržava sve etičke aspekte istraživanja, prije početka istraživanja potpisali su svi sudionici.

Randomizacija, generiranje sekvencija i zaslijepljivanje

Jednostavna randomizacija učinjena je generiranjem brojeva od 1 : 96 s pomoću Random Sequence Generatora, Randomness and Integrity Services Ltd. (www.randomization.com). Svaki generirani slučajni broj predstavljao je dodijeljenu intervenciju i bio je komparator na slučajan način. Istraživač i mikrobiolog bili su zaslijepljeni kad je riječ o dodijeljenom materijalu. No pacijenti nisu mogli biti zaslijepljeni zbog razlike između probiotičkoga jogurta i žvakaće gume.

Korišteni materijali bili su probiotički jogurt (Activia; Danone, Egipat) i žvakaća guma sa ksilitolom (Trident Original) – prikazani su u tablici 1. U interventnoj skupini sudionici su morali pojesti 100 grama probiotičkog jogurta 10 minuta nakon večere i ne četkati zube jedan sat[3]. U usporednoj skupini sudionici su dobili uputu da žvaču žvakaću gumu tri puta na dan po pet minuta nakon svakog obroka (3).

Sudionici u objema skupinama dobili su upute da tijekom istraživanja ne konzumiraju druge probiotičke proizvode i ne koriste se sredstvima za ispiranje usta. Svi su zamoljeni da tijekom istraživanja nastave sa svojim rutinskim navikama četkanja zuba i upotrebe zubnoga konca.

Broj SM-a u slini i interdentalnom plaku, pH sline i adherencija bakterija ocjenjivani su u različitim razdobljima – T0: prije intervencije, T1: poslije dva tjedna, T2: poslije mjesec dana i T3: poslije tri mjeseca.

Za određivanje broja *S. mutans* u slini ispitanici su dobili upute da ništa ne jedu i ne piju najmanje jedan sat prije uzimanja uzorka sline. Zamoljeni su također da isperu usta vodom prije skupljanja sline kako bi se izbjegla kontaminacija ostatcima hrane (8). Da bi se kontrolirale cirkadijalne varijacije, uzorci su prikupljeni između 10,00 i 11,00 sati.

Table 1 Materials' description, composition, lot number and manufacturers
Tablica 1. Opis materijala, sastav, serijski broj i proizvođač

Material • Materijal	Product description • Opis proizvoda	Composition • Sastav	Lot # • Serijski br.	Manufacturer • Proizvođač
Activia	Probiotic containing yogurt (100 gm) • Jogurt koji sadržava probiotik (100 g)	Skim milk, Ultrafiltered milk, Cream, Skim milk powder, Active probiotic culture (Bifidobacterium) and active bacterial cultures. • Obrano mlijeko, ultrafiltrirano mlijeko, vrhnje, obrano mlijeko u prahu, aktivna probiotička kultura (*Bifidobacterium*) i aktivne bakterijske kulture	NA • Nije primjenjivo	Danone, Obour city, kaliobeya, Egypt • Egipat https://www.dannon.com
Trident Original	Xylitol chewing gum • Žvakaća guma sa ksilitolom	Sorbitol, gum base, Xylitol, Glycerin, Natural and Artificial Flavor; less than 2% of: Acesulfame Potassium, aspartame, Butylated hydroxytoluene (to maintain freshness), Mannitol, Soy Lecithin and Sucralose. Phenylketonurics: contains phenylalanine • Sorbitol, gumena baza, ksilitol, glicerin, prirodna i umjetna aroma; manje od 2 %: acesulfam kalij, aspartam, butilirani hidroksitoluen (za održavanje svježine), manitol, sojin lecitin i sukraloza; fenilketonurici: sadržava fenilalanin	RK09419222V	Mondelez Global LLC, East Hanover, NJ 07936 USA • SAD https://www.tridentgum.com

water before collection of saliva to avoid the contamination of food debris (8). In order to control the circadian variations, the samples were collected between 10:00 am and 11:00 am.

The subjects were instructed to chew on a piece of paraffin wax for 5 minutes and the pooled saliva was expectorated into 15 ml sterile graduated falcon collection tube. Samples were mixed by vortexing for 30 seconds to disperse bacterial aggregates, and subsequently100 µL of each sample was diluted 1:10 with double-distilled water. After that, 10 µl aliquots of each diluted sample was transferred to the sterile *mitis salivarius* bacitracin agar. Inoculated culture media were incubated at 37°C in a candle extinction jar for 48 hours anaerobically in an atmosphere of 5-10% CO2 in a precision incubator.

For interdental plaque *Streptococcus mutans* count, samples were collected from four sites using separate dental floss threads for each site. These sites were: maxillary right molar – mesial surface, maxillary incisor – mesial surface, mandibular incisor – distal surface and mandibular left molar – distal surface. The plaque samples were then dispersed in a falcon tube containing saline. Samples were then prepared for culture in the same manner as salivary samples.

Salivary pH was assessed with the aid of a pH meter. The required minimum volume of saliva (2 ml) was placed in a sterile test tube so that the bulb of the measuring electrode could dip sufficiently into the saliva samples.

Measuring bacterial adherence was done by placing C-shaped cheek retractors to retract cheek, plaque disclosing tablets (Visuplac, Maquira, Brazil) were rubbed over the upper teeth to make dental plaque visible by staining the teeth areas that were covered by dental plaque. Standard photos were taken using digital camera mounted on a tripod. Digital image analysis used the Cool PHP Tools image color extract software by Kepler Gelotte (http://www.coolphptools.com/) to analyze plaque covering the upper six anterior teeth. Images were converted into pixels to calculate the percentage of the tooth covered with plaque divided by the total tooth area calculated by the software.

Statistical analysis

Statistical Analyses were performed using Medcalc software, version 19 for Windows (MedCalc Software Ltd, Ostend, Belgium). Data were explored for normality using the Kolmogorov-Smirnov test and the ShapiroWilk test. Continuous data were described using mean and standard deviation. An intergroup comparison between continuous data was performed using t-test, while intragroup comparison was performed using repeated measures ANOVA, and two-way ANOVA was used to test interaction of variables. A p-value less than, or equal to 0.05, was considered statistically significant, and all tests were two tailed.

Results

The intergroup comparison between both materials have shown non-significant differences within follow up periods; baseline and 4 weeks respectively (P = 0.9020, P = 0.0852), but there was a statistically significant difference at 2 weeks where probiotics showed higher level of reduction in MS count and 3 months where xylitol showed higher level of re-

Subjektima je naloženo da 5 minuta žvaču komadić parafinskoga voska, a slina je prikupljana u 15-mililitarsku sterilnu graduiranu epruvetu. Uzorci su 30 sekunda miješani vorteksiranjem da bi se raspršili bakterijski agregati, a zatim je 100 µL svakog uzorka razrijeđeno 1 : 10 s dvostruko destiliranom vodom. Nakon toga su alikvoti od 10 µL svakoga razrijeđenog uzorka prebačeni na sterilni agar *mitis salivarius bacitracin*. Inokulirani mediji kulture inkubirani su na 37 °C u posudi za gašenje svijeća 48 sati anaerobno u atmosferi od 5 do 10 % CO_2 u preciznom inkubatoru.

Za broj *S. mutans* u interdentalnom plaku uzorci su skupljeni s četiriju mjesta korištenjem odvojenih niti zubnoga konca za svako mjesto. Ta mjesta bila su maksilarni desni kutnjak – mezijalna površina, maksilarni sjekutić – mezijalna površina, mandibularni sjekutić – distalna površina i mandibularni lijevi kutnjak – distalna površina. Zatim su uzorci plaka raspršeni u epruveti Falcon koja je sadržavala fiziološku otopinu. Uzorci su nakon toga pripremljeni za kulturu na isti način kao i uzorci sline.

pH sline procijenjen je s pomoću pH-metra. Potrebni minimalni volumen sline (2 mL) stavljen je u sterilnu epruvetu kako bi se žarulja mjerne elektrode mogla dovoljno uroniti u uzorke sline.

Mjerenje adherencije bakterija obavljeno je postavljanjem retraktora obraza u obliku slova C, tablete za otkrivanje plaka (Visuplac, Maquira, Brazil) utrljane su preko gornjih zuba da bi postao vidljiv – naime, područja zuba koja su prekrivena zubnim plakom promijene boju. Standardne fotografije snimljene su digitalnim fotoaparatom postavljenim na stativ. Digitalna analiza slike provedena je s pomoću softvera za ekstrakciju boja Cool PHP Tools od Kepler Gelotte (http://www.coolphptools.com/) za analizu plaka koji prekriva šest gornjih prednjih zuba. Slike su pretvorene u piksele kako bi se izračunao postotak zuba prekrivenog plakom podijeljen s ukupnom površinom zuba koju je izračunao softver.

Statistička analiza

Statističke analize obavljene su u softveru Medcalc verzija 19 za Windowse (MedCalc Software Ltd, Ostend, Belgija). Normalnost distribucije podataka analizirana je Kolmogrov-Smirnovljevim i Shapiro-Wilkovim testom. Kontinuirani podatci opisani su korištenjem srednje vrijednosti i standardne devijacije. Međugrupna usporedba kontinuiranih podataka provedena je s pomoću t-testa, a unutargrupna usporedba ANOVA-om s ponovljenim mjerenjima. Za ispitivanje interakcije varijabli korištena je dvosmjerna ANOVA. P-vrijednost manja ili jednaka 0,05 smatrana je statistički značajnom i svi su testovi bili dvostrani.

Rezultati

Međugrupna usporedba između obaju materijala pokazala je neznatnu razliku unutar razdoblja praćenja; početna i poslije 4 tjedna (P = 0,9020, P = 0,0852), ali je postojala statistički značajna razlika poslije 2 tjedna kada su probiotici pokazali veće smanjenje broja MS-a i 3 mjeseca kada je ksilitol pokazao veće smanjenje broja MS-a od probiotika (P <

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duction in MS count than probiotics (P < 0.0001). Intragroup comparisons with xylitol chewing gum or probiotic yoghurt have shown statistically significant difference between different follow-up periods (P < 0.001). The results are presented in Table 2.

The intergroup comparison between both materials has shown a statistically significant difference within follow up periods; 2 weeks (P = 0.0021) where probiotics showed greater reduction in plaque bacterial count, 4 weeks and 3 months respectively (P < 0.0001 and P < 0.0001) where xylitol showed greater reduction in plaque bacterial count, but

0,0001). Unutargrupna usporedba u skupinama sa žvakaćom gumom sa ksilitolom i onih s probiotičkim jogurtom pokazala je statistički značajnu razliku između različitih razdoblja praćenja (P < 0,001). Rezultati su prikazani u tablici 2.

Međugrupna usporedba između obaju materijala pokazala je statistički značajnu razliku unutar razdoblja praćenja; poslije 2 tjedna (P = 0,0021) kada su probiotici pokazali veće smanjenje broja bakterija plaka, poslije 4 tjedna i 3 mjeseca (P < 0,0001 i P < 0,0001) kada je ksilitol pokazao veće smanjenje broja bakterija u plaku, ali nije bilo statistički značajne razlike na početku (P = 0,3297). Unutargrupna uspored-

Table 2 Mean and standard deviation of Log CFU/ml of salivary bacterial count
Tablica 2. Srednja vrijednost i standardna devijacija Log CFU/mL broja bakterija u slini

Intervention • Intervencija / Follow-up • Praćenje	Xylitol • Ksilitol		Probiotic • Probiotik		P value • P vrijednost
	Mean • Sredina	SD	Mean • Sredina	SD	
Baseline • Početak	6.5909	0.07892	6.5889	0.07314	P = 0.9020 NS
2 weeks • tjedna	6.4619	0.06707	6.2441	0.05879	P < 0.0001*
4 weeks • tjedna	5.5235	0.05586	5.5505	0.09162	P = 0.0852 NS
3 months • mjeseca	4.2242	0.06763	4.4078	0.1023	P < 0.0001*
P value • P vrijednost	P < 0.001		P < 0.001		

Table 3 Mean and standard deviation of Log CFU/ml of plaque bacterial count
Tablica 3. Srednja vrijednost i standardna devijacija Log CFU/mL broja bakterija u plaku

Intervention • Intervencija / Follow-up • Praćenje	Xylitol • Ksilitol		Probiotic • Probiotik		P value • P vrijednost
	Mean • Sredina	SD	Mean • Sredina	SD	
Baseline • Početak	4.5711	0.08206	4.5864	0.07065	P = 0.3297 NS
2 weeks • tjedna	4.2344	0.08567	4.1648	0.1263	P = 0.0021*
4 weeks • tjedna	3.3677	0.06156	3.7700	0.1774	P < 0.0001*
3 months • mjeseca	2.8785	0.1161	3.2799	0.09644	P < 0.0001*
P value • P vrijednost	P < 0.001		P < 0.001		

Table 4 Mean and standard deviation of pH
Tablica 4. Sredina i standardna devijacija pH vrijednosti

Intervention • Intervencija / Follow-up • Praćenje	Xylitol • Ksilitol		Probiotic • Probiotik		P value • P vrijednost
	Mean • Sredina	SD	Mean • Sredina	SD	
Baseline • Početak	7.3250	0.2817	7.4083	0.3093	P = 0.1709 NS
2 weeks • tjedna	7.3896	0.2707	7.4875	0.2523	P = 0.0700 NS
4 weeks • tjedna	7.4313	0.2502	7.5188	0.2358	P = 0.0811 NS
3 months • mjeseca	7.4812	0.2312	7.5583	0.2082	P = 0.0894 NS
P value • P vrijednost	P < 0.001		P < 0.001		

Table 5 Mean and standard deviation of plaque adherence percentage
Tablica 5. Srednja vrijednost i standardna devijacija postotka adherencije plaka

Intervention • Intervencija / Follow-up • Praćenje	Xylitol • Ksilitol		Probiotic • Probiotik		P value • P vrijednost
	Mean • Sredina	SD	Mean • Sredina	SD	
Baseline • Početak	56.1250	6.2284	58.4167	6.6070	P = 0.0836 NS
3 months • mjeseca	6.0833	1.6221	3.8333	1.7785	P < 0.0001*
P value • P vrijednost	P < 0.0001		P < 0.0001		

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there was no statistically significant difference at baseline (P = 0.3297). The intragroup comparison within xylitol chewing gum or probiotic yoghurt groups has shown a statistically significant difference between different follow-up periods (P < 0.001). The results are presented in Table 3.

The intergroup comparison between both materials has shown no statistically significant differences within follow up periods; baseline, 2 weeks, 4 weeks and 3 months respectively (P = 0.1709, P = 0.0700, P = 0.0811 and P = 0.0894). The intragroup comparisons within xylitol chewing gum or probiotic yoghurt groups have shown statistically significant differences between different follow-up periods (P < 0.001). The results are presented in Table 4.

The intergroup comparison between both materials has shown a statistically significant difference at 3 months (P < 0.0001), but there was no statistically significant differences at baseline (P = 0.0836). The intragroup comparison within xylitol chewing gum or probiotic yoghurt has shown statistically significant difference between different follow-up periods (P < 0.0001). The results are presented in Table 5.

Discussion

Dental caries is a chronic biofilm mediated disease caused by dysbiosis that manifests when the cariogenic oral pathogens dominate over the healthy commensals (9). Improvement of the oral flora is one of the effective strategies to prevent dental caries (10). Biofilm-targeted strategies to restore ecological symbiosis can either be biofilm inhibitory or biofilm modulating (9).

Inhibition of bacterial biofilm is achieved using antimicrobial agents that act against *Streptococcus mutans* such as xylitol. Xylitol, a sugar alcohol, has a unique inhibitory effect on glycolysis of MS where intracellular accumulation of xylitol-5-phosphate leads to reduced acid formation from glucose and a reduction in bacterial count in both plaque and saliva (11). However, unabsorbed xylitol can be fermented in the colon by bacteria, resulting in the production of a considerable amount of hydrogen that causes laxation and diarrhea (6). Therefore, the aim of the current study was to achieve a modality with suitable efficacy against MS but without these complications.

The biofilm modulating approach aims to restore and maintain oral homeostasis by enhancing the growth of healthy oral commensals, effectively attenuating the presence of pathogens, thereby maintaining a diverse symbiotic ecological microbiome (9). Probiotics have been confirmed to co-aggregate with MS and inhibit their growth by colonizing and competing with such oral pathogens for adhesion sites (12).

Preventive care in geriatric individuals must be simple and fast with techniques and materials adapted to their specific features. Since geriatric people lean towards a soft diet that is easier to chew, probiotic yogurt is ideal for them (13).

Measuring *Streptococcus mutans* in saliva is one of the most popular methods to recognize subjects at risk of dental caries. All the groups in the current study had similar salivary count of *Streptococcus mutans* at baseline so that the changes seen after the administration of the test products could be attributed to the use of these products.

ba u skupinama sa žvakaćom gumom sa ksilitolom i onih s probiotičkim jogurtom pokazala je statistički značajnu razliku između različitih razdoblja praćenja (P < 0,001). Rezultati se nalaze u tablici 3.

Međugrupna usporedba između obaju materijala nije pokazala statistički značajnu razliku unutar razdoblja praćenja; početna vrijednost poslije 2 tjedna, poslije 4 tjedna i poslije 3 mjeseca (P = 0,1709, P = 0,0700, P = 0,0811 i P = 0,0894). Unutargrupna usporedba u skupinama sa žvakaćom gumom sa ksilitolom i onih s probiotičkim jogurtom pokazala je statistički značajnu razliku između različitih razdoblja praćenja (P < 0,001). Rezultati su u tablici 4.

Međugrupna usporedba između obaju materijala pokazala je statistički značajnu razliku poslije 3 mjeseca (P < 0,0001), ali nije bilo statistički značajne razlike na početku (P = 0,0836). Unutargrupna usporedba u skupinama sa žvakaćom gumom sa ksilitolom i onih s probiotičkim jogurtom pokazala je statistički značajnu razliku između različitih razdoblja praćenja (P < 0,0001). Rezultati su prikazani u tablici 5.

Rasprava

Zubni karijes kronična je bolest posredovana biofilmom, a prouzročena je disbiozom koja se manifestira kada kariogeni oralni patogeni dominiraju nad zdravim komenzalima (9). Poboljšanje oralne flore jedna je od učinkovitih strategija u prevenciji karijesa (10). Strategije usmjerene na biofilm za obnavljanje ekološke simbioze mogu biti inhibicijske ili modulirajuće (9).

Inhibicija bakterijskoga biofilma postiže se korištenjem antimikrobnih sredstava koja djeluju protiv *S. mutans*, kao što je ksilitol. Ksilitol, šećerni alkohol, ima jedinstveni inhibitorni učinak na glikolizu MS-a gdje unutarstanično nakupljanje ksilitol-5-fosfata smanjuje stvaranje kiseline iz glukoze i broj bakterija u plaku i slini (11). No neapsorbirani ksilitol može fermentirati u debelome crijevu, što rezultira proizvodnjom znatne količine vodika koji uzrokuje laksaciju i proljev (6). Zato je cilj istraživanja bio postići modalitet s odgovarajućom učinkovitošću protiv MS-a, ali bez navedenih komplikacija.

Pristup koji modulira biofilm ima za cilj obnoviti i održavati oralnu homeostazu povećanjem rasta zdravih oralnih komenzala, učinkovito umanjujući prisutnost patogena, čime se održava raznolik simbiotski ekološki mikrobiom (9). Potvrđeno je da se probiotik koagregira s MS-om i inhibira njegov rast kolonizacijom i natjecanjem s takvim oralnim patogenima za mjesta adherencije (12).

Preventivna skrb, kad je riječ o gerijatrijskim pacijentima, mora biti jednostavna i brza s tehnikama i materijalima prilagođenima njihovim specifičnim potrebama. Budući da stariji ljudi preferiraju mekana jela koja je lakše žvakati, za njih je idealan probiotički jogurt (13).

Mjerenje *S. mutans* u slini jedna je od najpopularnijih metoda za prepoznavanje subjekata s rizikom od karijesa. Sve skupine u ovom istraživanju imale su na početku sličan broj *S. mutans* u slini, tako da se promjene uočene poslije primjene ispitivanih proizvoda mogu pripisati njihovoj upotrebi.

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In the current study, the statistically significant reduction in the level of salivary MS count over all the examined follow up periods in xylitol gum group was due to xylitol inhibitory effect on glycolysis and subsequent intracellular accumulation of xylitol-5-phosphate leading to a dramatic reduction in MS count (11, 14).

The probiotic yogurt group showed statistically significant reduction in the level of salivary MS count over all the examined follow up periods of the study since probiotic bacteria produce antibacterial agents against oral pathogens such as hydrogen peroxide, organic acids and bacteriocins (15). They are also effective in regulating local and systemic immune systems. They compete with the pathogenic MS bacteria and impede their viability in saliva (16).

The results of the intergroup comparison showed a statistically significant difference between two materials in a two week period of time where probiotics showed higher levels of reduction in the MS count. This could be due to the rapid antibacterial action of probiotics in saliva by co-aggregation with MS and fast elimination (15). This was followed by statistically non-significant difference between both materials after one-month timeframe. This was due to increase in level of MS reduction in xylitol group between a two week time period and a four week period of time compared to probiotics. The results also showed a statistically significant difference between both groups where xylitol showed higher level of reduction in MS count than probiotics after three months. This could be due to long term action of xylitol in addition to the washing action of chewing gum which facilitates bacterial clearance from saliva (17).

Regarding the count of plaque bacteria, statistically significant reduction in the level of MS between follow up periods in xylitol gum group was found owing to the ability of xylitol to disturb MS energy production and reduce the adhesion of these microorganisms to the teeth surface by diminishing the production of extracellular polysaccharides (18, 19).

In the current study, the statistically significant reduction in the level of plaque bacterial count between follow up periods in probiotic yogurt group could be explained by the local biofilm effect of probiotics on MS relying on bacterial co-aggregation, bacteriocin and hydrogen peroxide production, competition for adhesion sites, and competition for nutrients with such pathogenic bacteria (20).

The results of intergroup comparison showed statistically significant difference between the two materials in a two week time period where probiotics showed greater reduction in plaque bacterial count. This could be due to the rapid ability of probiotic in preventing the adherence of other bacteria to the tooth surface by modifying the protein composition of the salivary pellicle on tooth surface via binding to it, thus causing its degradation (21).

However, the intergroup comparison showed statistically significant difference between the two materials in a four week period of time and a three month period of time where xylitol showed greater reduction in plaque bacterial count. This is attributed to the inability of microorganisms of dental plaque to ferment xylitol leading to inhibition of their

U ovom istraživanju statistički značajno smanjenje razine MS-a u slini tijekom svih ispitivanih razdoblja praćenja u skupini sa žvakaćim gumama sa ksilitolom bilo je posljedica inhibitornog učinka ksilitola na glikolizu i naknadnoga intracelularnoga nakupljanja ksilitol-5-fosfata, što je drastično smanjilo broj MS-a (11, 14).

Skupina s probiotičkim jogurtom pokazala je statistički značajno smanjenje razine MS-a u slini tijekom svih ispitivanih razdoblja praćenja jer probiotičke bakterije proizvode antibakterijske agense protiv oralnih patogena kao što su organske kiseline vodikova peroksida i bakteriocini (15). Također su učinkoviti u regulaciji lokalnoga i sustavnoga imunosnog sustava. Natječu se s patogenim bakterijama MS-a i ometaju njihovu održivost u slini (16).

Rezultati usporedbe među skupinama pokazali su statistički značajnu razliku između dvaju materijala tijekom dva tjedna kada su probiotici pokazali veću razinu smanjenja broja MS-a. To bi mogla biti posljedica brzoga antibakterijskoga djelovanja probiotika u slini koagregacijom s MS-om i brzom eliminacijom (15). Nakon toga slijedila je statistički neznatna razlika između obaju materijala nakon jednomjesečnoga vremenskog okvira. To se dogodilo zbog povećanja razine smanjenja MS-a u skupini sa ksilitolom između dva tjedna i četiri tjedna u usporedbi s probioticima. Zatim su rezultati pokazali statistički značajnu razliku između obiju skupina jer je ksilitol poslije tri mjeseca postigao veću razinu smanjenja broja MS-a od probiotika. To bi mogla biti posljedica dugotrajnog djelovanja ksilitola, uz djelovanje žvakaće gume koja olakšava uklanjanje bakterija iz sline (17).

Kad je riječ o broju bakterija plaka, pronađeno je statistički značajno smanjenje razine MS-a između razdoblja praćenja u skupini sa žvakaćim gumama sa ksilitolom zbog svojstva ksilitola da poremeti proizvodnju energije MS-a i smanji adherenciju tih mikroorganizama na površinu zuba smanjenjem proizvodnje ekstracelularnih polisaharida (18, 19).

U ovom istraživanju statistički značajno smanjenje razine broja bakterija plaka između razdoblja praćenja u skupini s probiotičkim jogurtom moglo bi se objasniti lokalnim učinkom biofilma probiotika na MS koji se oslanja na koagregaciju bakterija, proizvodnju bakteriocina i vodikova peroksida, konkurenciju za mjesta adherencije i natjecanje za hranjive tvari s takvim patogenim bakterijama (20).

Rezultati usporedbe među skupinama pokazali su statistički značajnu razliku između dvaju materijala tijekom dva tjedna kada su probiotici pokazali veće smanjenje broja bakterija u plaku. To bi mogla biti posljedica brze sposobnosti probiotika u sprječavanju adherencija drugih bakterija na površinu zuba modificiranjem proteinskog sastava pelikule sline na površini zuba vezivanjem na nju uzrokujući njezinu degradaciju (21).

Međutim, usporedba među skupinama pokazala je statistički značajnu razliku između dvaju materijala u razdobljima od četiri tjedna i tri mjeseca kada je ksilitol postigao veće smanjenje broja bakterija u plaku. To se pripisuje nesposobnosti mikroorganizama iz zubnoga plaka da fermentiraju ksilitol, što inhibira njihov rast i svojstvo ksilitola da proizvodi unutarstanične vakuole unutar *S. mutans* i tako prouzroči degradaciju njihove stanične membrane (18). Kombinacija

growth as well as the ability of xylitol to produce intracellular vacuoles inside *Streptococcus mutans* causing degradation of their cell membrane (18). Combining mechanical cleaning and salivary stimulation effect of chewing gum gives further benefits compared to the antibacterial effect alone.

The statistically significant increase in salivary pH in xylitol gum group along the follow-up periods could be attributed to the inability of MS to ferment xylitol, and therefore cannot produce acids that cause drop in the pH of the oral cavity. In addition, the action of stimulating salivary flow by chewing gum aids in increasing the action of saliva buffering systems (22).

The statistically significant increase in salivary pH in probiotic yogurt group along the follow-up periods was due to immunomodulatory action of probiotics by increasing the number of secretory IgA (sIgA) producing cells found in saliva. These sIgA producing cells inhibit the function of the glucosyltransferase enzyme from *Streptococcus mutans*, and therefore diminishing its acid production ability preserving a homeostatic environment (23).

In the current study, Xylitol gum led to statistically significant decrease in plaque adherence to tooth surface over a three month intervention period by inhibiting the glycolysis process in cariogenic bacteria by competing with the phosphofructokinase enzyme leading to reduction in insoluble polysaccharides which plays an important role in bacterial adherence (24).

The statistically significant reduction in bacterial adherence in probiotic group after three months of consumption of probiotic yogurt was due to production of antioxidants by probiotic bacteria that utilize the free electrons required for mineralization of plaque, thus inhibiting plaque formation (25). Probiotics also compete with bacteria for binding sites on host tissues causing disruption of plaque biofilm formation (26).

The present study is the first study investigating the beneficial oral effect of using probiotics on geriatric people. For geriatric population, yogurt is a very simple and easy way to deliver an antibacterial agent. Therefore, according to the results of the current study, probiotic yogurt can be used as an alternative to xylitol in enhancing the oral condition and prevention from caries.

Conclusions

Under the conditions used in this clinical trial, it could be concluded that probiotic yogurt is an effective antibacterial agent against salivary and plaque bacteria in geriatric patients and can be used as a caries prevention means.

This is the first study investigating the beneficial oral effect of using probiotics on geriatric patients. For geriatric population, yogurt is a very simple and easy way to deliver an antibacterial agent. Therefore, according to the results of the current study, probiotic yogurt can be used as an alternative to xylitol in enhancing the oral condition and prevention from caries.

More studies on geriatric population are required to investigate probiotic yogurt antibacterial effects, and long term follow up is required in future studies to identify the emergence of any bacterial resistance to probiotics.

mehaničkoga čišćenja i učinka žvakaće gume na stimulaciju sline daje dodatne prednosti u usporedbi sa samim antibakterijskim učinkom.

Statistički značajno povećanje pH sline u skupini sa žvakaćim gumama sa ksilitolom tijekom razdoblja praćenja moglo bi se pripisati nesposobnosti MS-a da fermentira ksilitol i stoga ne može proizvesti kiseline koje uzrokuju pad pH u usnoj šupljini. Osim toga, stimuliranje protoka sline žvakaćim gumama pomaže povećati djelovanje puferskih sustava sline (22).

Statistički značajno povećanje pH sline u skupini s probiotičkim jogurtom tijekom razdoblja praćenja posljedica je imunomodulatornog djelovanja probiotika povećanjem broja stanica koje proizvode sekretorne IgA-e (sIgA) koji se nalaze u slini. Te stanice koje proizvode sIgA inhibiraju funkciju enzima glukoziltransferaze iz *S. mutans* i zato smanjuju njegovo svojstvo proizvodnje kiseline čuvajući homeostatsko okruženje (23).

U ovom istraživanju žvakaća guma sa ksilitolom statistički je značajno smanjila adherenciju plaka na površinu zuba tijekom tri mjeseca intervencije inhibirajući proces glikolize u kariogenim bakterijama natječući se s enzimom fosfofruktokinaze, što rezultira smanjenjem netopljivih polisaharida koji su važni u adherenciji bakterija (24).

Statistički značajno smanjenje prianjanja bakterija u probiotičkoj skupini nakon tromjesečne konzumacije probiotskog jogurta, uzrokovano je proizvodnjom antioksidansa u probiotskim bakterijama koje se koriste slobodnim elektronima potrebnima za mineralizaciju plaka i tako inhibiraju njegovo stvaranje (25). Probiotici se također natječu s bakterijama za mjesta vezanja na tkivima domaćina uzrokujući poremećaj u stvaranju biofilma plaka (26).

Ovo je prvo istraživanje koje se bavi povoljnim oralnim učinkom probiotika na gerijatrijsku populaciju. Za gerijatrijsku populaciju jogurt je vrlo jednostavan i lagan način za isporuku antibakterijskoga sredstva. Zato se, prema dosadašnjim rezultatima istraživanja, probiotički jogurt može upotrijebiti kao alternativa ksilitolu u poboljšanju oralnoga stanja i prevenciji karijesa.

Zaključak

U uvjetima korištenima u ovom kliničkom istraživanju može se zaključiti da je za gerijatrijske pacijente probiotički jogurt djelotvorno antibakterijsko sredstvo protiv bakterija sline i plaka i može se primijeniti za prevenciju karijesa.

Ovo je prvo istraživanje koje ispituje povoljan intraoralni učinak probiotika na gerijatrijsku populaciju. Za starije ljude jogurt je vrlo jednostavan i lagan način za isporuku antibakterijskoga sredstva. Zato se, prema dosadašnjim rezultatima istraživanja, probiotički jogurt može koristiti kao alternativa ksilitolu u poboljšanju oralnoga stanja i prevenciji karijesa.

Potrebno je više istraživanja o gerijatrijskoj populaciji kako bi se istražio antibakterijski učinak probiotičkog jogurta, a dugotrajno praćenje potrebno je u budućim istraživanjima da bi se identificirala pojava bakterijske rezistencije na probiotike.

Conflicting Interest

The author declares that there is no conflict of interest.

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Author's contribution: **H.M.A.G.** - Operator, data entry & corresponding author; **M.S.I.** - Data monitoring & auditing: **O.O.S.** - Sample size calculation and statistical analysis

Sukob interesa

Autori nisu bili u sukobu interesa.

Etička pravila i odobrenje institucijskoga Odbora za reviziju

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Doprinos autora: **H. M. A. G.** – Kreator studije, unos podataka i autor za dopisivanje; **M. S. I.** - Praćenje i revizija podataka: **O. O. S.** - Izračun veličine uzorka i statistička analiza

Sažetak

Svrha istraživanja: Procijeniti kod gerijatrijskih pacijenata učinak probiotičkih bakterija u jogurtu prema broju bakterija *Streptococcus mutans* (SM), adherenciji plaka i pH sline u usporedbi sa žvakaćom gumom koja sadržava ksilitol. **Materijali i metode:** Ukupan broj od 96 gerijatrijskih pacijenata s visokim rizikom od pojave karijesa randomiziran je u dvije jednake skupine (n = 48). Skupina 1 (interventna skupina) konzumirala je probiotički jogurt (Activia, Danone) jedanput na dan, a skupina 2 (kontrolna skupina) dobivala je žvakaću gumu sa ksilitolom (Trident original) tri puta na dan. Primarni ishod bio je broj bakterija *Streptococcus mutans* u slini, a sekundarno se želio doznati broj tih bakterija u interdentalnom plaku, pH sline i adherencija SM-a. **Rezultati:** Za broj bakterija *Streptococcus mutans* u slini i plaku postignuto je statistički značajno smanjenje njihove razine tijekom svih razdoblja praćenja u skupini s probiotičkim jogurtom te u skupini sa žvakaćom gumom koja sadržava ksilitol. Međugrupna usporedba za broj SM-a u slini pokazala je statistički značajnu razliku između dvaju materijala u razmaku od dva tjedna i tri mjeseca, a nije bilo statistički značajne razlike između obaju materijala u razdoblju od jednog mjeseca. Rezultati pH sline pokazali su statistički značajno povećanje vrijednosti u objema skupinama tijekom razdoblja praćenja. Rezultati bakterijske adherencije pokazali su statistički značajno smanjenje u objema skupinama. **Zaključak:** Probiotički jogurt učinkovito je antibakterijsko sredstvo protiv bakterija u slini i plaku kod gerijatrijskih pacijenata.

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