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Original Article

Effects of Short-term Consumption of Probiotic Yogurt on Streptococcus Mutans and lactobacilli Levels in 18-30 Years Old Students with Initial Stages of Dental Caries in Ahvaz City

Ahmad Zare Javid[*1], Essam Amerian[2], Leila Basir[3], Alireza Ekrami[4], Mohammad Hosein Haghighi-zadeh[5]

1- Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

2- M.Sc Student in Nutrition Sciences, Arvand International Division of Medical Sciences, Abadan, Iran.

3- Dept.of Pediatric Dentistry, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

4- Dept.of Medical Laboratory Sciences, School of Paramedicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

5- School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

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ABSTRACT

Background and Objectives: Dental caries, caused by oral microbial flora, is considered as one of the most common infectious diseases in human. The aim of this study was to determine the effect of short-term consumption of probiotic yogurt containing *bifidobacterium lactis* on salivary *streptococcus mutans* and *lactobacilli* in students with initial stages of dental caries.

Materials and Methods: 66 students (18-30 years old) with initial stages of dental caries were selected in this single blind randomized clinical trial. The subjects were randomly assigned into two groups: intervention group received 300g/d probiotic yogurt, and control group received 300 g/d conventional yogurt for 2 weeks. Un-stimulated fasting saliva sample was collected pre- and post-intervention. Bacterial counting was performed for salivary *streptococcus mutans* and *lactobacilli*. *Salivarius Mitis* agar and *Rogosa agar* were used as culture media for *streptococcus mutans* and *lactobacilli,* respectively.

Results: The number of *streptococcus mutans* in saliva was significantly reduced in the intervention group post-intervention (P<0.001); however, it was not changed in the control group (P=0.71). *Streptococcus mutans* was also significantly lower in the intervention group compared with the control group post-intervention (P<0.001). Although salivary *lactobacilli* was reduced significantly in both groups post-intervention (P<0.001), this reduction was not significantly greater in the intervention group compared with the control group (P=0.594).

Conclusions: It is suggested that consumption of probiotic yogurt may be useful to prevent the progression of dental caries.

Keywords: Probiotic yogurt, Streptococcus mutans, Lactobacilli, Bifidobacterium lactis, Tooth decay

Introduction

Dental caries is one of the common oral health problems worldwide. It is a multi-factorial disease of calcified tooth developed by demineralization of inorganic parts (enamel) and loss of organic material of tooth (1). There are several factors involved in developing dental caries, including host factors, environmental factors and micro-organisms (2). Tooth decay develops as a result of irreversible dissolution of enamel minerals due to acid production by certain bacteria. The plaque on tooth surface is cariogenic bacteria attached to bacteria communities (3).

***Address for correspondence:** Ahmad Zare Javid, Professional Assistant Member of Nutrition and Metabolic diseases Research Center, Jondishapour University of Medical Sciences, Ahvaz, Iran. E-mail address: ahmaddjavid@gmail.com

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Oral cavity is a combination of dynamic, open, complex and unique environment. This cavity plays an important role in defense systems against the organisms such as cariogenic bacteria. Systemic secretion of saliva is an important factor in maintaining optimal oral health. Saliva composition, generally, consists of about 99.4% water and 0.6% organic and mineral matter (4).

The oral cavity contains a wide range (200-300 species) of bacteria. However, only a small number of specific bacteria species have cariogenic properties (5). Cariogenic bacteria have the ability to produce a high acidity pH by producing lactic acid through the fermentation of carbohydrates. *S. mutans* has been known as the main cause of tooth decay while various *lactobacilli* types are associated with progression of the lesions, especially in dentin (6,7).

S. mutans is an effective micro-organism in dental caries development. The reason is the ability to produce an insoluble substance in water called "branched-glucan" or "mutant" that facilitates *S. mutans* establishment in dental biofilm. This may subsequently lead to an acidogenic environment of oral cavity with low pH (less than 5.5) and rapid metabolism of carbohydrates (7).

Lactobacilli (with colonization capability) forms about 1% of human mouth flora. These bacteria are often found in dairy products; however, there is no evidence that the species found in oral cavity are originated from frequent consumption of dairy products (leading to their temporary colonization in the mouth cavity). Also available evidence indicates that oral cavity is not considered as a permanent residence for these species of bacteria (6). DMFT (Decayed, Missed, Filled Teeth) index is the most commonly used indicator in order to detect the risk of dental caries. DMFT, as a simple, fast and functional index in dentistry, has been widely used for decades (8).

The effect of probiotics on dental caries and its related risk factors have been evaluated in several experimental studies. In addition, probiotic treatment is considered as an important alternative therapy technique for replacement of pathogen micro-organism. It is suggested that probiotics may be useful for reducing dental plaques that initiate the progress of decay (9,13,14,15,21,22,24).

There is disagreement about the minimum concentration of probiotic bacteria used in dairy products. However, generally, it is suggested that the minimum concentration of 10^6- 10^7 CFU/ml is needed to have probiotic effects (10).

In one study carried out by Tahmourespour et al. in 2008, the standard strain of ATCC 35668 *S. mutans* with 40 strains of *S. mutans* and other oral *Streptococci* were isolated from dental plaque of subjects with caries. The results showed that reducing the adhesion of cariogenic pathogens can be an effective approach to reduce the probable carcinogenicity of oral streptococci (11).

The results of a randomized double-blinded cross-over study performed by Caglar et al. in 2005 showed that consuming yogurt containing *B. lactis* in young adults for two weeks led to a temporary reduction in the number of *S. mutans* during the intervention period and a few days after intervention. So it was concluded that probiotics should be used constantly in order to be effective (12).

In another randomized double-blinded cross-over intervention carried out by Caglar et al. in 2008, consuming100 ml (53 g) ice-cream containing *B. lactis Bb-12* reduced the salivary S. mutants levels significantly after 10 days in young adults; but the salivary *lactobacilli* levels did not change (13).

Regarding the limited information about the effects of probiotic products on oral health and conflicting results of previous studies, as well as the importance of oral health, this study aimed to evaluate the effects of short-term consumption of probiotic yogurt containing *B. lactis* on salivary *S. mutans* and *lactobacilli* in the students with initial stages of dental caries in Ahvaz Jundishapur University of Medical Sciences (AJUAMS).

Materials and Methods

In this single blind randomized clinical trial (Ethical Code: AJUMS. REC. 28. 2014; registration code in Iran clinical trials: IRCT. 1 N2014062518240) 66 students (18-30 years old) were selected from Ahvaz Jundishapur University of Medical Sciences. The total number of required subjects for this study was calculated using the below formula:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \left[P_1(1-P_1) + P_2(1-P_2)\right]}{(P_1 - P_2)^2}$$

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A questionnaire including demographic features and oral health data was completed, and a preliminary examination in terms of detecting DMFT (inclusion criteria of 1-3 with initial stages of dental caries) was performed. Individuals with more than 3 decayed teeth or active caries, using mouth wash, brush more than twice daily, smokers, and people who have consumed antioxidant supplements or anti-inflammatory drugs or antibiotics or any probiotic products routinely during the last month, were excluded.

All subjects were asked to maintain their normal diet, brush their teeth 2 times a day and do not use xylitol gums and probiotic products at the same time.

A consent form was obtained from all candidates participating in the study.

The subjects were randomly dividedin2 groups; intervention group and control group (n=33). The anthropometric measurements were done at baseline. A fasting un-stimulated saliva sample (2ml) was collected at baseline and post-intervention for microbial counting. The collected saliva samples were immediately transported in sterile containers to the Laboratory of Microbiology in order to do the experiments.

The subjects in the intervention group and control group consumed 300 mg of domestically produced probiotic yogurt and the conventional yogurt, respectively daily for 2 weeks. The yogurts were distributed in 2 stages (the first stage was before beginning of the intervention and the next stage was one week after starting the intervention) in order to monitor and ensur the consumption of yogurts).

The probiotic yogurt used in the present study contained a minimum count of 10^6CFU/ml of *B. lactis*. Both probiotic and conventional yogurts contained similar percentage of fat (3%). Also both products had the same trading brand.

Microorganisms and cultivation: For microbiological analysis, 20µl of the saliva sample was spread on *Salivarious Mitis agar* (Hi-Media, India) for total *S. mutans* counting. Furthermore, 20µl of the saliva sample was spread on *Rogosa agar* media (Hi-Media, India) in order to count total *lactobacilli*. The microbial cultivation was done using streak-***plate*** method. Both groups of plates were placed in an anaerobic condition at 37°C for 3 days. 5% CO_2 was added to *lactobacilli* culture media to improve the growth of colonies. The number of colony forming units was counted using a colony counter expressed as per milliliter (CFU/ml).

Statistical Analyses: Independent sample T-test was used to compare the mean differences of bacterial counts between the two groups, and paired T-test was used to compare mean differences within the groups. The analyses were done using the SPSS software (ver. 19, Chicago, IL, USA). The confidence interval was considered at 95%.

The data of bacterial counting were displayed as mean±standard deviation (Mean±SD). In order to determine whether the data have a normal distribution, a visual examination of the data and a goodness of fit test (Kolmorogov-Smirnoff) were conducted. A histogram was also used to confirm the normal distribution of the data.

Results

66 subjects including 42 females (62%) and 24 males (38%) completed the study. The mean± standard deviation for age, weight and DMFT was 22.1 ± 3.1 years, 67.85 ± 3.7 kg and 2.47 ± 0.38, respectively. All subjects had normal body mass index (BMI: 22.86±2.47 kg/m^2). Demographic and anthropometric data are shown in Table 1

.

Table 1. Demographic and anthropometric characteristics of the subjects

Characteristics	Intervention group (n=33)	Control group (n=33)	*P-value*
Men	13	11	0.8
Women	20	22	0.79
Weight (kg)	68.2±4.4*	66.3±4.1*	0.74
Age (years)	21.6±2.5*	22.4±3.8*	0.77
DMFT	2.2±0.7*	2.2±0.9*	0.91
BMI (kg / m^2)	22.77±2.37	22.54±2.25*	0.87

Refers to values, which are the mean ±SD.
P-values were calculated using Kolmogorov-Smirnov's normality test.

The mean number of *S. Mutans* in saliva in the intervention group was significantly greater than in the control group (2624.24 vs. 1440.90 CFU/ml; P=0.0001) at baseline. Moreover, the mean number of *lactobacilli* in the intervention group did not

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significantly differ from the control group (11963.63CFU/ml vs. 13383.3CFU/ml; P=0.13) at baseline.

The results showed that salivary *S. Mutans* were significantly decreased in the intervention group post-intervention (P<0.001). A similar trend was observed for *lactobacilli*, but this decrease was not significant (P=0.594). According to Table 2, the mean number of *S. mutans* was significantly lower in the intervention group compared with the control group post-intervention (621.21±69.99 and 1093.93±68.38 CFU/ml; P <0.001). Furthermore, the mean number of *lactobacilli* was significantly lower in the intervention group compared with the control group post-intervention (1898.48± 132.08 and 4345.45 ± 267 CFU/ml; P=0.01).

Table 2. Mean ± SD for *S. mutans* and *lactobacilli* within- and between-group comparative analysis

Variables	Time	I (CFU/ml) n= (33)	C (CFU/ml) n=(33)	*P-value***
S. mutans	baseline	2624.24± 137.73	1440.90± 138.83	0.001
	2 weeks	621.21± 69.99	1093.93± 68.38	0.001
	Mean change (95% CI)	2003.03± 124.04	346,96± 108.46	
	*P-value**	0.001	0.071	
Lactobacilli	baseline	11963.63± 8246.7	13380.30± 9274.35	0.06
	2 weeks	1898.48± 1825.08	4345.45± 2672	0.001
	Mean change (95% CI)	10065.15± 7767.96	9034,84± 7842.78	
	*P-value**	0.001	0.594	

I: Bacterial count for intervention group
C:Bacterial count for Control group
*P <0.05 was considered as significant using Paired T-test.
**P <0.05 was considered as significant using Independent T-test

Discussion

This study aimed to investigate the effect of probiotic yogurt on cariogenic bacteria in patients with initial stages of dental caries. The results showed that the short-term probiotic yogurt consumption significantly reduced the number of *S. Mutans* in saliva. These findings are consistent with the results of several other studies including Nase et al. (2001), Caglar et al. (2008), and Cildir et al. (2009) (13, 14, 15).

The tooth decay process is well-understood now, and is a dynamic balance between the pathological factors such as acidogenic bacteria, reduced functional properties of saliva (in order to neutralize the high acidity), and lack of protective factors including protein, calcium, phosphate and fluoride for dental caries (16).

When exposed to high concentrations of carbohydrates in the diet, *S. mutans* and *lactobacilli* produce lactic acid, which can lead to demineralization of enamel and dentin and play a key role in the beginning and progression of dental caries (12, 17). It seems that probiotics may prevent the formation of dental plaque either directly through adhering to tooth surface or indirectly through neutralizing free electrons (18).

As different types of probiotic bacteria have several effects on the growth of cariogenic bacteria, species such as *lactobacilli* and *bifidobacterium* have long history of safety for commercial or treatment uses. However, some species of *lactobacilli* are acidogenic, and it seems that they may be associated with the development of dental caries (19). A probiotic microorganism should have the ability to stick the

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teeth in order to join the bacterial biofilm in teeth, and to prevent the progression of pathogenic bacteria in order to create positive effects on oral health (20).

According to some studies, *bifidobacterium* can reduce dental caries among the people who do not have active caries (11). In Nase and Ahola's studies, the type of probiotic used was *L. rhamnosus* (13 , 21). In Nikawa's study (2004), the inhibiting effect of *L. reuteri* on the growth of *S. mutans* was evaluated (22). In Montalto's study (2004), the type of used probiotic was a combination of 20 species of *lactobacilli* including 18% *L. acidophilus*, 12% *L. thermophilus*, 12% *L. rhamnosus*, 10% *L. bulgaricus* and 48% *L. casei*. The presence of *lactobacilli* as probiotic in the yogurt samples used in this study showed increased levels of *lactobacilli* in the saliva samples within 2 weeks (23). In a study carried out by Chuang et al. (2010), it was found that consuming *GMNL-33 L. paracasei* tablets 3 times a day increased the levels of saliva *lactobacilli* after 2 weeks (24). The findings of a clinical trial carried out by Moini et al. (2013) on 30 dentistry students with healthy teeth showed that in the group who received probiotic yogurt containing *L. acidophilus* for 3 weeks, saliva *S. mutans* level was reduced in 18 subjects while there was no change in 7 subjects and 5 subjects (1).

The results of Lesan et al.'s study (2013) on 32 healthy subjects with *S. mutans* and 28 healthy subjects with *lactobacilli* revealed that short-term consumption of probiotic yogurt did not change *S. mutans* populations, but significantly reduced the level of salivary *lactobacilli*. The reducing effects of using *lactobacilli*-derived probiotics on salivary *S. Mutans* levels was observed in some interventions (14, 21, 23). However, diverse results were reported also by other studies (22, 24).

The type of probiotic used, period of intervention, the study design, and characteristics of the target group may be factors causing such diversity in the results in different studies (25).

In the present study, the probiotic yogurt contained *B. lactis*. According to some studies, it seems that reduced levels of *S. mutans* in saliva post-intervention is not dependent on the administration vehicle of probiotics, such as milk, cheese, yogurt or lozenge (26, 27).

There are several studies focused on reducing *S. mutans* aiming to dental caries prevention. In addition, many of previous studies have been conducted on healthy individuals in terms of oral health or patients with periodontal diseases.

This study showed that short-term (2 weeks) consumption of probiotic yogurt can prevent the progression of dental caries in those who are at risk of active dental caries through reducing the levels of *S. mutans* and *lactobacilli* (cariogenic bacteria) in saliva.

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References

1. Moini P, Jamei N, Mohamadi M, SHafizadeh N, Valaei N, Rahbar M, et al . Effect of a probiotic yogurt produced in Iran on the salivary counts of Streptococcus mutans. J Res Dent Sci. 2013; 10 (2):73-82. [In Persian].
2. James B. Sumitt, William Robbins, Richard Shwartz: Fundamentals of Operative Dentistry. 3rd. Quintessence. Carol Stream. 2006; ch: 3, 51-66.
3. Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 2001; 65(10): 1028-37.
4. Teresa A. Marshall, Ph.D., R.D., University of Iowa College of Dentistry Department of Preventive and Community Dentistry 329 DSB N Iowa City, 2010, IA 522242-1010.
5. Walter J. Loesche, Microbiology of Dental Decay and Periodontal Disease, Chapter 99, Edited by Samuel Baron, University of Texas Medical Branch at Galveston, Galveston, Texas, Galveston (TX): University of Texas Medical Branch at Galveston; 1996 ISBN-10: 0-9631172-1-1.
6. Teanpaisan R, Dahlen G. Use of polymerase chain reaction techniques and sodium dodecyl sulfate-polyacrylamide gel electrophoresis for differentiation of oral

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Lactobacillus species. Oral Microbiol Immunol. 2006; 21(2):79-83.

7. Hoorizad M, Mirhashemi route M, Rahpeyma R, Salehi M, Zavareian S. Effect of probiotic yoghurt on the salivary PH of orthodontic patients- A clinical trial study. J Res Dent Sci. 2013; 10 (3):155-159. [In Persian].
8. Bratthall D. Estimation of global DMFT for 12-year-olds in 2004. Int Dent J. 2005; 55(6):370-2.
9. Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, et al. Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol 2002 40(3): 1001-9.
10. Mortazavian AM, Sohrabvandi S. Probiotics and probiotic dairy products. 1st ed. Tehran: Eta Co; 2006. pp. 20-63.
11. Tahmourespour A, Kasra-Kermanshahi R, Salehi R, Nabi-nejad A. The effect of lactobacillus fermentum ATCC9338 as a probiotic on the adhesion of oral streptococci in vitro. Iran J Med Microbiol. 2008; 2 (1):45-51. [In Persian].
12. Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifido- bacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontol Scand. 2005 63(6): 317-20.
13. Caglar E, KuscuOO ,Selvi KS, Kavaloglu CS, Sandalli N, Twetman S. Short-term effect of ice-cream containing Bifidobacteriumlactis Bb-12 on the number of salivary mutans streptococci and lactobacilli. Acta Odontol Scand. 2008 66(3): 154-8.
14. Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries Res. 2001; 35(6): 412-20.
15. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary Mutant streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. Eur J Orthod 2009; 31:407-11.
16. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen L, Floyd E Dewhirst FE et al. Bacteria of dental caries in primary and permanent teeth in children and young adults, J Clin Microbiol . 2008 April; 46(4): 1407-17.
17. Simark-Mattsson C, Emilson CG, Hakansson EG, Jacobsson C, Roos K, Holm S. Lactobacillus-mediated interference of mutans streptococci in caries-free vs. caries-active subjects. Eur J Oral Sci 2007; 115(4): 308-14.
18. Jagat Bhushan, Sanjay Chachra, Probiotics – Their role in prevention of dental caries , Journal of Oral Health Comm Dent 2010 4(3):78-82.
19. Bonifait L, Chandad F, Grenier D. Probiotics for oral health: Myth or reality? J Can Dent Assoc 2009; 75(8): 585-90.
20. Arathi R. Principles and practice of pedodontics.2th Ed. New Dehli: Japaee Brothers Medical Publisher Ltd. 2008; 11:166-170.
21. Ahola AJ, Yli-Knuuttila H, Suomalainen T, Poussa T, Ahlstrom A, Meurman JH, et al. Short-term consumption of probiotic-containing cheese and its effect on dental caries risk factors. Arch Oral Biol 2002; 47(11): 799-804.
22. Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, Santoro L, Cuoco L, Manna A, Gasbarrini G. Probiotic treatment increases salivary counts of lactobacilli: A Double – Blind , Randomized, Controlled Study. Digestion 2004; 69:53-56.
23. Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K, et al. Lactobacillus reuteri in bovine milk fermented decreases the oral carriage of mutans streptococci. Int J Food Microbiol 2004; 95(2): 219-23.
24. Chuang LC, Huang CS, Ou-Yang LW, Lin SY. Probiotic Lactobacillus paracasei effect on cariogenic bacterial flora. Clin Oral Investig 2010; 423-9.
25. Lesan S, Rahbar M, Valaei N, Abbasi Vardoogh S, Abbasi Vardoogh M. Effect of probiotic yoghurt on salivary Streptococcus mutans and Lactobacillus. Pejouhesh. 2013; 37 (3) :151-157. [In Persian].
26. Meurman JH, Stamatova I. Probiotics: Contributions to oral health. Oral Dis 2007; 13(5): 443-51.
27. Anderson MH, Shi W. A probiotic approach to caries management. Pediatr Dent 2006; 28(2): 151-3.
28. Aguilera Galaviz LA, Premoli G, Gonzalez A, Rodriguez RA Caries risk in children: Determined by levels of mutans streptococci and Lactobaccilus. J ClinPediatr Dent 2005; 29(4):329-33.
29. Petti S, Tarsitani G, D'Arca A. S. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Arch Oral Biol2001; 46, 705-12.
30. Ferrazzano GF, Cantile T, Sangianantoni G, Amato I, Ingenito A. The effects of short-term consumption of commercial yogurt on salivary mutans streptococci and lactobacilli counts: an in vivo investigation. European Journal of Clinical Nutrition 65, 1170-72 (October 2011).

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