Dental Research Journal

Original Article

Effect of milk and yogurt on *streptococcus sobrinus* counts and caries score in rats

Maryam Ghasempour[1], Ramazam Rajabnia[2], Manouchehr Ashrafpour[3], Atiyyeh Ehsani[1], Ali Akbar Moghadamnia[4], Samane Gharekhani[1], Elaheh Ferdosi Shahandashti[2], Meghdad Bagheri[2]

[1]Department of Pediatric Dentistry, School of Dentistry, Babol University of Medical Sciences, [2]Department of Microbiology, School of Medicine, Babol University of Medical Sciences, [3]Department of Physiology, School of Medicine, Babol University of Medical Sciences, [4]Department of Pharmacology and Toxicology, School of Medicine, Babol University of Medical Sciences, Babol, Iran

ABSTRACT

Background: An anti-cariogenic diet containing probiotics can be effective in caries prevention. This animal study compared the effects of milk and yogurt on *Streptococcus sobrinus* counts and caries score.
Materials and Methods: A total of 36 male rats were infected with *S. sobrinus* (27,607) and divided into three groups. Group A and B received 200 mL of milk and 100 g of yogurt per day, respectively, and a control group received 2.5 mL of NCP number 2 diet twice daily for 21 days. After killing the animals, their lower left jaws were removed and sonicated to quantify the colonies of *S. sobrinus*. Dental caries was scored using Keyes technique. Data were analyzed using ANOVA and Kruskal-Wallis, Mann-Whitney and Wilcoxon-Signed Rank tests. Statistical significance was set at $P < 0.05$.
Results: The mean (±standard error of the mean) of *S. sobrinus* colonies in the milk, yogurt and control groups were determined at 119666.67 (±20733), 46416.666 (±12846) and 163,250 (±33493), respectively. Microbial counts decreased in the yogurt group compared with the milk and control groups ($P = 0.004$ and $P = 0.000$; respectively). There were significant differences between caries scores of smooth surfaces in the milk and yogurt groups compared with the control group ($P = 0.000$ and $P = 0.000$, respectively). Both milk and yogurt significantly reduced caries score of fissured surfaces compared with controls ($P = 0.004$ and $P = 0.000$, respectively).
Conclusion: Considering the limitations of this study, yogurt administration reduces *S. sobrinus* counts. In addition, yogurt and milk regimens reduce the caries scores of smooth and fissured surfaces.

Key Words: Dental caries, milk, *Streptococcus sobrinus*, yogurt

Received: November 2013
Accepted: July 2015

Address for correspondence: Dr. Ramazan Rajabnia, Department of Microbiology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.
E-mail: Ramazan69@yahoo.com

INTRODUCTION

Tooth decay is the most common chronic disease of early childhood. Although it can be preventable, dental caries is considered a multi-factorial infectious and transmissible disease.[1] The most acceptable theory of caries development is the chemicoparasitic theory in which the presence of cariogenic bacteria, susceptible host and fermentable carbohydrate as a substrate for microbial action are critical; therefore, chemical and mechanical microbial plaque removal, as well as sugar discipline, are commonly advised.[2,3]

Access this article online

Website: www.drj.ir
www.drjjournal.net
www.ncbi.nlm.nih.gov/pmc/journals/1480

How to cite this article: Ghasempour M, Rajabnia R, Ashrafpour M, Ehsani A, Moghadamnia AA, Gharekhani S, *et al*. Effect of milk and yogurt on *streptococcus sobrinus* counts and caries score in rats. Dent Res J 2015;12:569-73.

Diet plays a key role in caries development. In other words, an anticariogenic diet can be effective in caries prevention.[4] The low cariogenic potential of food is attributed to high protein and mineral content as well as moderate fat content and high buffering capacity facilitating the saliva action to protect the teeth.[5] Dairy products such as milk and yogurt are excellent foods containing protein, providing essential amino acids and organic nitrogen. They also contain calcium, phosphate, casein, and lipids, which are considered factors with anticariogenic effects. These ingredients modulate the acidity of saliva and plaque, causing the remineralization of early carious lesions, in addition to having some degrees of antibacterial properties.[5]

In addition, yogurt contains probiotics, living beneficial microorganisms with an inhibitory effect on pathogenic bacteria.[6] Yogurt and other fermented milk-based products have been demonstrated to be beneficial for general health, especially because of their probiotic content. They were proposed as an alternative to manage many disorders such as infectious diseases, cancers and gastrointestinal problems in particular.[7] Because of so many interactions known between different species of microorganisms, it has been shown that the growth of pathogenic strains can be inhibited by interfering with colonization and their interaction with nonpathogenic neighbors.[8] In this respect, probiotic therapy refers to selected removal of pathogenic bacteria by nonpathogenic competitors.[9] The most common probiotic bacteria used for caries control are some species of *Bifidobacteria* and *Lactobacilli*, which are tested against *Streptococcus mutans*.[10]

Mutans streptococci are the major pathogenic bacteria in the caries process. This group mainly includes *S. mutans* and *Streptococcus sobrinus*, which are responsible for caries development in both animals and humans.[5] *S. sobrinus* is a primary bacterial pathogen on smooth surface dental caries. It presents in 43-60% of plaque cultures of children with early childhood caries.[11] It is supposed that the inhibition of these microorganisms leads to caries prevention.

This comparative study was carried out to evaluate the effects of milk and yogurt on *S. sobrinus* counts and caries score in rats.

MATERIALS AND METHODS

The present study was approved by the Ethic Committee of Babol University of Medical Sciences, and all experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23 revised in 1996).

Thirty-six male Wistar rats aged 19 days and weighing 25 ± 5 g were used in this study. The *S. sobrinus*-free rats were screened by culturing the saliva samples on streptococcus selective agar medium. Then, they were infected with *S. sobrinus* PTCC 27607 (Persian Type Culture Collection, Tehran, Iran) under the cariogenic diet 2000 sterilized by deionized distilled water with 10% sucrose *ad libitum* to establish the infection for 6 days.[12] Plating of oral swabs showed the rats were successfully infected with *S. sobrinus*.

All the animals were then anesthetized with intraperitoneal injection of a mixture of ketamine (8 mg/kg) and xylazine (2.5 mg/kg)[13] and their submandibular and parotid salivary glands were surgically removed when aged 25 days. After surgery, they were divided into three experimental groups (12 in each group). Group A and B were respectively fed *ad libitum* with 200 mL of milk and 100 g of yogurt daily and the control group received gavage of 2.5 mL of liquid diet NCP number 2 twice daily.[14] Milk and yogurt were chosen in the same brand, with similar fat, protein and carbohydrate contents. The animals were housed one per cage (42 cm × 26 cm) and maintained on a 12-h light/12-h dark cycle (lights on at 6.00 a.m.), at a temperature of 21°C ± 1°C and relative humidity of 50-70%. They had free access to water and diet related to each group.

After 21 days, the animals were sacrificed, and their lower left jaws were removed and suspended in 10 mL of 0.9% normal saline.[15] The samples were sonicated (BAN Delin Sonoplus, Germany) for 30 s in duty cycle 1 × 10. One loop was inoculated on streptococcus selective agar medium (Merck, Germany) and incubated at 37°C for 2 days for detecting and counting the colonies of *S. sobrinus*.

Dental caries was detected under a stereomicroscope at magnification of ×40 (Micro-Optic Industrial Group Company, China) and then scored using the Keyes technique.[16] Microbiological data and the total number of lesions on smooth and fissured surfaces were assessed separately.

Data were analyzed using SPSS 18 (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis analysis of variance was used to compare the levels of *S. sobrinus*. Ordinal caries scores were analyzed using

Kruskal-Wallis, Mann-Whitney and Wilcoxon-Signed Rank methods. Statistical significance was defined at $P < 0.05$.

RESULTS

The mean (±standard error of the mean) of *S. sobrinus* colonies in milk, yogurt and control groups were estimated at 119666.67 (±20733), 46416.666 (±12846) and 163,250 (±33493), respectively. A significant reduction in *S. sobrinus* counts was found in the yogurt group compared with the milk and control groups ($P = 0.004$ and $P = 0.000$; respectively), but milk consumption did not significantly reduce *S. sobrinus* counts. *S. sobrinus* counts are displayed in Figure 1.

There were significant differences between all the groups in caries scores of smooth surfaces ($P = 0.000$).

Consumption of milk and yogurt resulted in a significant decrease in dental caries scores on smooth surfaces compared with the controls ($P = 0.000$ and $P = 0.000$, respectively) but there was no significant difference between yogurt and milk groups. The caries scores of smooth surfaces are displayed in Table 1.

In relation to caries scores of fissured surfaces, a significant difference was shown between all the groups ($P = 0.000$). Both milk and yogurt significantly decreased the caries scores of fissured surfaces compared to the controls ($P = 0.004$ and $P = 0.000$, respectively) while there were no significant differences between yogurt and milk groups. The caries scores of fissured surfaces are presented in Table 2.

Figure 2 illustrates the rats' second molars, which were sectioned mesio-distally at the central fissure to find carious lesion based on the Keyes technique [Figure 2].

Wilcoxon-Signed rank did not reveal any significant differences between caries scores on smooth and fissured surfaces in all the groups.

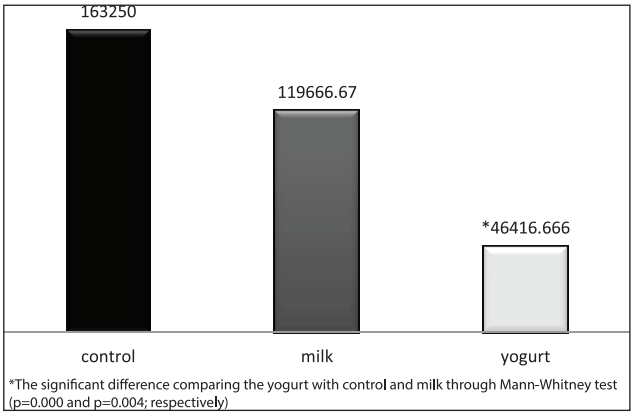

Figure 1: *Streptococcus sobrinus* counts in experimental groups.

DISCUSSION

This study evaluated *S. sobrinus* counts and caries scores by administration of milk and yogurt in rats. The results indicated that consumption of yogurt had an inhibitory effect on *S. sobrinus*, consistent with observations made by Caglar *et al*. and Petti *et al*.[17,18]

It suggested that the yogurt affects the oral microflora because of its probiotic microorganisms.[19] These beneficial bacteria are suggested as the nonpathogenic competitors against cariogenic bacteria.[9] It is supposed that interactions between microbial species prevent

Table 1: Caries scores on smooth surfaces

Group	First molar	Second molar	Third molar	Total	*P* value‡
Control	5.5	4.5	3.5	13.5	0.000
Milk	3	1.5	1	5.5*	
Yogurt	2	1	1	5.5*	

‡Significant difference between caries scores of molars in all groups through Kruskal-Wallis; *Significant difference compared to the controls through Mann-Whitney ($P = 0.000$).

Table 2: Caries scores on fissured surfaces

Group	First molar	Second molar	Third molar	Total	*P* value‡
Control	4	3	2	9	0.000
Milk	2	2	1	4*	
Yogurt	2	2	1	3**	

‡Significant difference between caries scores of molars in all the groups through Kruskal-Wallis test; *Significant difference between the control and milk groups through Mann-Whitney test ($P = 0.004$); **Significant difference between the control and yogurt groups through Mann-Whitney test ($P = 0.000$).

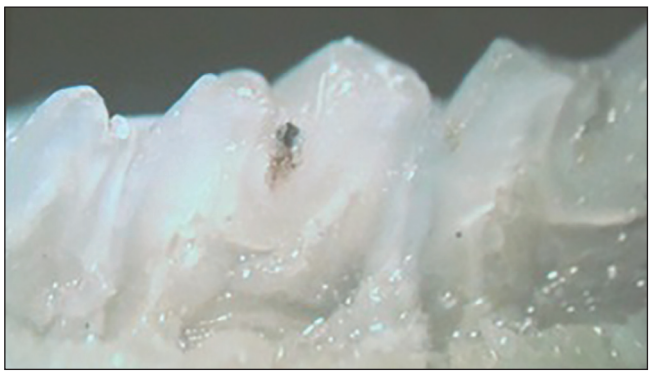

Figure 2: The rat second molar sectioned mesio-distally at the central fissure.

colonization of pathogenic bacteria by interfering with cellular adhesion and biofilm formation.[9,20] Furthermore, some probiotic strains produce and secrete organic acids, hydrogen peroxide and bacteriocins, making the ecosystem unsafe for pathogenic neighbors.[20]

Probiotic microorganisms naturally exist in some dietary products such as yogurt. In addition, they may be added to dairy products, chewing gums, food supplements, pills, etc.[21] However, probiotics-containing dairy products appear to be the most natural and the best approach for probiotic therapy.[22] Not only the probiotics content but also the other components such as high mineral and protein and moderate lipid contents in these products play a major role in caries prevention.[5]

Milk consumption did not significantly decrease *S. sobrinus* counts compared to the control group. The results of two studies conducted by Engström *et al*. and Lexner *et al*.[23,24] are consistent with the present study. This outcome can be probably explained by considering the lack of probiotics in milk, since the efficacy of probiotics-containing milk in reduction of caries-associated bacteria has been previously demonstrated.[25,26]

The anticariogenic effect of probiotic milk containing *Lactobacillus rhamnosus* GG was evaluated on children aged 1-6 years during 7 months. It was found that the incidence of tooth decay may decrease by a long-term daily consumption of this product.[25]

Additionally, it was observed that consumption of milk and yogurt significantly decreased caries scores of smooth and fissured surfaces compared with the control group. In this regard, Tanaka *et al*.[27] found that the frequent use of yogurt (more than or up to 4 times a week) might be associated with a lower incidence of dental caries in children. In addition, Levy *et al*.[28] showed that regular administration of milk had a protective effect on primary teeth.

It is suggested that milk ingredients such as fat, casein, calcium and phosphate have a protective effect against tooth decay.[29] Aimutis[30] reported that the casein phosphopeptides (CPPs) and glycomacropeptide in dairy products prevented demineralization as well as stimulated remineralization of tooth enamel. The above-mentioned effect was also demonstrated by Ferrazzano *et al*.[31] about CPPs of yogurt.

The CPPs stabilize high concentrations of calcium and phosphate together with fluoride ions on the enamel surface. These ions are freely bioavailable to diffuse into early carious lesions, thereby effectively enhancing remineralization.[32] They also have a great capacity to neutralize the acid produced by cariogenic bacteria to prevent demineralization.[33]

This study was designed as an animal study on rats mainly due to ethical considerations existing in human studies. In spite of natural differences between rats and humans, based on previous studies,[17,18,27,28] it seems that if this project had been performed on humans, similar findings would have been achieved. However, further studies on humans, comparing the anticaries properties of dairy products on *S. mutans* and *S. sobrinus* are recommended.

CONCLUSION

This study showed that yogurt administration significantly reduced *S. sobrinus* counts. Although it was not statistically significant, *S. sobrinus* counts dropped by consumption of milk. Additionally, taking yogurt and milk reduced the caries scores of smooth and fissured surfaces significantly. These results confirmed the anticariogenic capacity of yogurt and milk.

ACKNOWLEDGEMENTS

We are pleased to the staff of microbiology and histology departments of the Babol University of Medical Sciences for their scientific co-operations and thanks Dr. Feizi for her practical guidance.

Financial support and sponsorship

This project was financially supported by Babol University of Medical Sciences.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

1. Kiessling G, Schneider J, Jahreis G. Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. Eur J Clin Nutr 2002;56:843-9.
2. Marshall TA, Levy SM, Broffitt B, Warren JJ, Eichenberger-Gilmore JM, Burns TL, *et al*. Dental caries and beverage consumption in young children. Pediatrics 2003;112:e184-91.
3. Harris N, Garcia-Godoy F, Nathe CN. Primary Preventive Dentistry. 7th ed. USA: Pearson Education; 2009. p. 37.
4. Dean JA, Avery DR, McDonald RE. Dentistry for the Child and Adolescent. 9th ed. USA: MOSBY; 2011. p. 193.
5. Casamassimo PS, Fields HW, McTigue DJ, Nowak A. Pediatric Dentistry: Infancy Through Adolescence. 5th ed. USA: Elsevier Saunders; 2013. p. 178, 288.

6. Tabbers MM, Chmielewska A, Roseboom MG, Boudet C, Perrin C, Szajewska H, *et al.* Effect of the consumption of a fermented dairy product containing Bifidobacterium lactis DN-173 010 on constipation in childhood: A multicentre randomised controlled trial (NTRTC: 1571). BMC Pediatr 2009;9:22.
7. Flichy-Fernández AJ, Alegre-Domingo T, Peñarrocha-Oltra D, Peñarrocha-Diago M. Probiotic treatment in the oral cavity: An update. Med Oral Patol Oral Cir Bucal 2010;15:e677-80.
8. He X, Lux R, Kuramitsu HK, Anderson MH, Shi W. Achieving probiotic effects via modulating oral microbial ecology. Adv Dent Res 2009;21:53-6.
9. Stamatova I, Meurman JH. Probiotics: Health benefits in the mouth. Am J Dent 2009;22:329-38.
10. Agarwal E, Bajaj P, Guruprasad CN, Naik S, Pradeep AR. Probiotics: A novel step towards oral health. AOSR 2011;1:108-15.
11. Choi EJ, Lee SH, Kim YJ. Quantitative real-time polymerase chain reaction for *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque samples and its association with early childhood caries. Int J Paediatr Dent 2009;19:141-7.
12. Rosalen PL, Bowen WH, Pearson SK. Influence of fluoride co-cystallized with sugar on caries development in desalivated rats. Arch Oral Biol 1997;42:317-22.
13. Shahidi S, Komaki A, Mahmoodi M, Lashgari R. The role of GABAergic transmission in the dentate gyrus on acquisition, consolidation and retrieval of an inhibitory avoidance learning and memory task in the rat. Brain Res 2008;1204:87-93.
14. Bowen WH, Amsbaugh SM, Monell-Torrens S, Brunelle J, Kuzmiak-Jones H, Cole MF. A method to assess cariogenic potential of foodstuffs. J Am Dent Assoc 1980;100:677-81.
15. Peres RC, Coppi LC, Volpato MC, Groppo FC, Cury JA, Rosalen PL. Cariogenic potential of cows', human and infant formula milks and effect of fluoride supplementation. Br J Nutr 2009;101:376-82.
16. Keyes PH. Dental caries in the molar teeth of rats. II. A method for diagnosing and scoring several types of lesions simultaneously. J Dent Res 1958;37:1088-99.
17. Caglar E, Sandalli N, Twetman S, Kavaloglu S, Ergeneli S, Selvi S. Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and *Lactobacilli* in young adults. Acta Odontol Scand 2005;63:317-20.
18. Petti S, Tarsitani G, Simonetti D'Arca A. Antibacterial activity of yoghurt against viridans streptococci *in vitro*. Arch Oral Biol 2008;53:985-90.
19. Levy SM, Warren JJ, Broffitt B, Hillis SL, Kanellis MJ. Fluoride, beverages and dental caries in young children. J Dent 2010;38:579-83.
20. Bonifait L, Chandad F, Grenier D. Probiotics for oral health: Myth or reality? J Can Dent Assoc 2009;75:585-90.
21. Twetman S, Stecksén-Blicks C. Probiotics and oral health effects in children. Int J Paediatr Dent 2008;18:3-10.
22. Lodi CS, Manarelli MM, Sassaki KT, Fraiz FC, Delbem AC, Martinhon CC. Evaluation of fermented milk containing probiotic on dental enamel and biofilm: *In situ* study. Arch Oral Biol 2010;55:29-33.
23. Engström K, Petersson LG, Sjöström I, Twetman S. Composition of the salivary microflora during habitual consumption of fluoridated milk. Acta Odontol Scand 2004;62:143-6.
24. Lexner MO, Blomqvist S, Dahlén G, Twetman S. Microbiological profiles in saliva and supragingival plaque from caries-active adolescents before and after a short-term daily intake of milk supplemented with probiotic bacteria — A pilot study. Oral Health Prev Dent 2010;8:383-8.
25. Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä 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;35:412-20.
26. Juneja A, Kakade A. Evaluating the effect of probiotic containing milk on salivary mutans streptococci levels. J Clin Pediatr Dent 2012;37:9-14.
27. Tanaka K, Miyake Y, Sasaki S. Intake of dairy products and the prevalence of dental caries in young children. J Dent 2010;38:579-83.
28. Levy SM, Warren JJ, Broffitt B, Hillis SL, Kanellis MJ. Fluoride, beverages and dental caries in the primary dentition. Caries Res 2003;37:157-65.
29. Grenby TH, Andrews AT, Mistry M, Williams RJ. Dental caries-protective agents in milk and milk products: Investigations *in vitro*. J Dent 2001;29:83-92.
30. Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr 2004;134:989S-95.
31. Ferrazzano GF, Cantile T, Quarto M, Ingenito A, Chianese L, Addeo F. Protective effect of yogurt extract on dental enamel demineralization *in vitro*. Aust Dent J 2008;53:314-9.
32. Reynolds EC. Calcium phosphate-based remineralization systems: Scientific evidence? Aust Dent J 2008;53:268-73.
33. Kumar VL, Itthagarun A, King NM. The effect of casein phosphopeptide-amorphous calcium phosphate on remineralization of artificial caries-like lesions: An *in vitro* study. Aust Dent J 2008;53:34-40.