

Comparative Study of Kefir Yogurt-Drink and Sodium Fluoride Mouth Rinse on Salivary Mutans Streptococci

¹Maryam Ghasempour, ²Seyyed Ali Asghar Sefidgar, ³Ali Akbar Moghadamnia, ⁴Reza Ghadimi
⁵Samane Gharekhani, ⁶Leila Shirkhani

ABSTRACT

Aim: The level of mutans streptococci (MS) is reduced by regularly consumption of probiotic Kefir drink. The present study aiming at answer to this question 'may the Kefir drink be considered an alternative to fluoride rinse?'

Materials and Methods: Twenty-two healthy volunteers aged 22-32 years with good oral hygiene participated in this study. None of participants had received any antibiotic or antimicrobial agent or professional fluoride therapy within the last month. None of them exhibited active caries lesions. Smokers and subjects who have chewing gums xylitol habit or who were undergoing orthodontic treatment were excluded. In a cross over design with 2 parallel groups, people in group A for 2 weeks received 100 ml of Kefir drink per day, then avoided brushing, eating and drinking for an hour. Simultaneously, people in group B were administered 0.05% sodium fluoride rinse in the same manner. After washout period, people in groups A and B respectively used 0.05% sodium fluoride rinse and Kefir drink. Saliva sampling was performed before and after interventions. The acidity and the count of MS were assessed and data were analyzed statistically.

Result: There was no significant difference between base line pH values of saliva in study groups ($p > 0.05$). Although, a significant difference was shown between MS counts before and after interventions ($p \leq 0.0001$ and $p \leq 0.0001$ for NaF and Kefir respectively), but the equal inhibitory effect was found between study groups ($p > 0.05$).

Conclusion: Kefir drink can inhibit salivary MS as well the sodium fluoride rinse. This beverage may be considered an alternative for fluoride rinse.

Clinical significance: Probiotics containing dairy products was known with anticariogenic properties. These agents may be used in caries control strategies adjunctively.

Keywords: Fluoride mouth rinse, Kefir, Mutans streptococci.

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INTRODUCTION

Oral cavity has been known as a complex nest for many of microorganisms are competing with each other to obtain food, shelter and growth opportunities. By understanding the interactions between them, pathogenic strains can be inhibited or selectively removed indirectly.¹ In term, this approach is named 'Bacteriotherapy' aiming at control decay by reducing or inhibiting cariogenic bacteria.²

The most important caries causing bacteria are mutans streptococci (MS) species. This group includes *Streptococcus mutans* and *Streptococcus sobrinus* that are responsible for occlusal and smooth dental surfaces caries respectively.³

Historically fluoride has been considered as a major cariostatic element that primarily affects smooth surfaces caries.³ It is applied in various forms and doses. One of the most common applications of fluoride is mouth rinse. It is found that a long-term use of daily fluoride rinse can reduce level of salivary MS species in addition to remineralization of initial caries lesions.⁴⁻⁶ Besides professional dental services, fluoride mouth rinse is suggested for high-risk dental patients, such as orthodontic patients, pregnant or new mothers and adolescents who suffering rampant caries.⁷⁻⁹

It is interested that increasing the application of fluoridated dental care products, resulted in a decreasing rate of decay in children, while dental caries in adolescents especially on occlusal surfaces has remained high.¹⁰

It seems that when the parental supervision on their teenager's oral hygiene is removed, bad dietary habits such as frequently intake of snacks and some soft drinks as well as reluctance or neglect in dental self-care or application of the fluorinated products by the juveniles are some of main factors contributing in high incidence of dental caries in this group.³

^{1,4,5}Assistant Professor, ²Associate Professor, ³Professor
⁶Non-faculty Investigator

^{1,5}Department of Pediatric Dentistry, Babol University of Medical Sciences, Iran

^{2,6}Department of Mycology, Babol University of Medical Sciences, Iran

³Department of Pharmacology, Babol University of Medical Sciences, Iran

⁴Department of Community Medicine, Babol University of Medical Sciences, Iran

Corresponding Author: Samane Gharekhani, Department of Pediatric Dentistry, Ganj Afrooz Street, Babol University of Medical Sciences, Iran, e-mail: sgharekhani@gmail.com

Unfortunately, in spite of good attempts were made to preserve oral hygiene, dietary pattern, is less considered by patients or even dentists. It is necessary to advise patients about good dietary habits to prevent caries. One of the excellent food sources in this field is probiotic fermented dairy products. Application of the Probiotic products or in term, bacteriotherapy is an alternative approach to regulate the ecology of oral cavity by reducing the number of cariogenic bacteria. The word 'probiotic' against 'antibiotic' has been introduced to inhibit, reduce or selectively removal of the pathogenic bacteria as well as to prevent the emergence of resistant strains.¹¹

One of the probiotic dairy sources, the probiotic drink Kefir, is popular in Middle East. This drink is produced by fermentation of the Kefir grains containing probiotic lactobacilli and yeasts. This beverage is full of vitamin K, B₁, B₂, calcium, folic acid and amino acids that is used to promote general health and to control of many diseases.¹²

The authors think that, beside the good taste and availability, this fact that people don't look to drink as a drug, is the advantage of this product.

Previously, a randomized, controlled study has demonstrated the inhibitory effect of Kefir drink on MS species.¹³ Under the assumption that adding Kefir drink to diet as well the sodium fluoride mouth rinse can reduce the number of MS species in saliva, this study is planned to compare the inhibitory effect between probiotic drink Kefir and sodium fluoride (NaF) mouth rinse on salivary mutans streptococci species.

MATERIALS AND METHODS

Subjects

A total of 22 healthy volunteers (11 males and 11 females) aged 22-32 years participated in this study. The informed consent was obtained from each subject. Also, the study protocol was approved by the ethics committee of the Babol University of Medical Sciences, Babol, Iran and it was registered in the Iranian Registry of Clinical Trials (IRCT) system with registration number IRCT201204119271N2. All participants had good oral hygiene and had not received any antibiotic or antimicrobial agent or professional fluoride therapy within the last month. None of them exhibited active or untreated caries lesions. Smokers and subjects whit chewing gums xylitol habit or who were undergoing orthodontic treatment were excluded from present study.

Study Design

This study was designed as a cross over randomized, controlled trial with two parallel groups. Following the 'run-in' period lasted 7 days that the participants were instructed not to use any probiotics products, people in group A for

2 weeks swished and drank 100 ml of Kefir drink per day, then avoided brushing, eating and drinking for an hour (n = 11). Simultaneously, people in group B swished their mouth in the same method by 0.05% NaF mouth rinse (n = 11). After 4 weeks (wash-out) period, people in groups A and B respectively used 0.05% NaF mouth rinse and Kefir drink in the cited manner. Totally, all 22 participants received both Kefir drink and NaF mouth rinse. Also, all of them were brushing their teeth with fluoride-containing toothpastes during the study while the use of other fluoridated products was avoided.

Kefir Yogurt-Drink Preparation

The homemade Kefir drink was used in this study. After boiling milk (1.5% fat, Pegah Dairy Co, Iran) and cooling it at room temperature, 5% of Iranian Kefir grains (containing *Lactobacillus casei* subsp. *Pseudo plantarum* and *Saccharomyces cerevisiae* and pH = 4) were added. The fermentation was done at 25°C for 48 hours, and then the Kefir grains were separated by filtering.¹²

Clinical Examination, Saliva Sampling

A dentist examined the subject's oral health using World Health Organization (WHO) criteria to rule out the presence of untreated active caries lesion. Unstimulated saliva sample was collected from each person before and after every intervention to measure its pH by pH indicator paper (ARAK Chemical Co, Iran) and count of MS. To minimize circadian rhythm effects, saliva sampling was done in the morning. The subjects were ordered not to eat or drink for an hour before the saliva sampling.

Laboratory Tests

The counts of mutans streptococci species were evaluated by culturing method. One loop of 10⁻³ dilution of saliva inoculated on blood agar plate (Merk, Germany) at 37°C for 48 hours. For different cultured colonies in size and shape, several biochemical tests, such as Gram staining, Catalase test, Resistance to Optochin (Sigma-Aldrich, USA) and Bacitracin discs (Padtan teb, Iran), Hydrolysis of Esculin (Himedia, India) and fermentation of the manitol and sorbitol (Sigma-Aldrich, USA) were done. Based on Bergy's manual, the colonies similar to isolated MS were counted.¹⁴

Statistical Analysis

Student t-test was used to compare the MS counts between two groups. The test was also used to evaluate changes in pH values of saliva before and after interventions. The differences between data of groups were considered statistically significant at p-value under 0.05.

Table 1: Mean values of mutans streptococci species CFU/ml of saliva, before and after interventions

Groups		Pre-intervention (10 ⁵ CFU/ml of saliva)	Post-intervention (10 ⁵ CFU/ml of saliva)	Delta (10 ⁵ CFU/ml of saliva)	p-value
NaF mouth rinse	Mean	5.375	2.632	*2.743	≤0.0001
	SD	1.687	1.203	1.370	
Kefir drink	Mean	5.282	2.975	*2.261	≤0.0001
	SD	1.644	1.264	1.588	

*Non-significant difference between two groups (p > 0.05)

Result

There was no significant difference between pH values of saliva before and after interventions (p > 0.05) (Fig. 1).

Although, a significant difference was shown between salivary MS counts before and after interventions (p ≤ 0.0001 and p ≤ 0.0001 for NaF and Kefir; respectively), but the equal inhibitory effect was found between study groups (p > 0.05) (Table 1).

DISCUSSION

In the present study, Kefir yogurt-drink containing probiotic *Lactobacillus casei* subsp. *Pseudo plantarum* and *Saccharomyces cerevisiae* could reduce salivary MS species count as well sodium fluoride mouth rinse. Unfortunately, less data exist on the efficacy of Kefir in caries prevention and such a comparison had not been done previously. The only *in vivo* study about the effect of Kefir drink on cariogenic bacteria count was carried out by Cugulo et al. They showed that 3 weeks consumption of Kefir yogurt-drink, 2 times per day could be effective on reduction of MS count.¹³ As previously mentioned, some of milk based products, such as yogurt and yogurt-drink contain probiotic microorganisms that are found a main factor in caries prevention.^{13,15} Probiotics can inhibit their pathogenic neighborhoods by interfering with biofilm formation, cellular adhesion and colonization.¹⁵⁻¹⁸

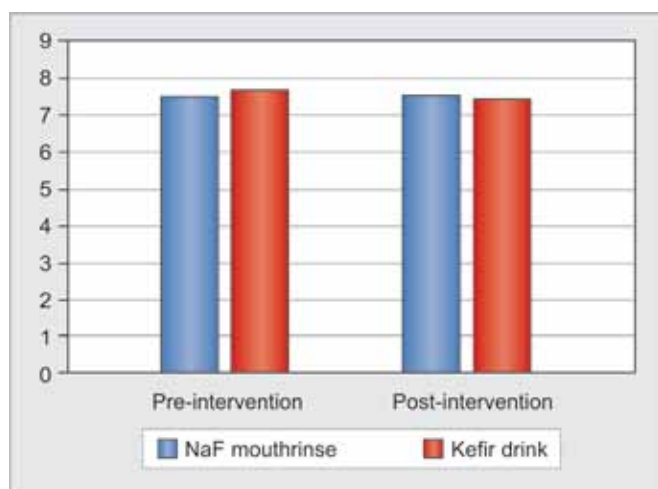


Fig. 1: Mean values of pH of saliva before and after interventions

Interestingly, the remineralization potential was also reported for probiotic milk-based products.^{19,20} It seems that the presence of calcium and other constituents which may protect enamel against dental pathogen is responsible for remineralization process.²¹ Thereafter, in view of authors, probiotic products may be considered an alternative for fluoride mouth rinse especially in young children who not be allowed to use fluoride mouth rinse.

Considering the low pH of Kefir yogurt-drink, there was a concern if it may drop pH of saliva under critical value. In this study after 2 weeks daily consumption of Kefir yogurt, no significant change was found in pH of saliva. Indeed, the buffering capacity of milk and saliva may regulate the pH.²¹

Since lactobacilli are responsible for caries development in deep lesions,²²⁻²⁴ there is another concern if probiotic lactobacilli may increase other salivary lactobacilli count. Cugulo et al were demonstrated reduction of lactobacilli as well as mutans streptococci species in saliva with intake of Kefir drink for 3 weeks.¹³ Unlikely, Montalto et al were reported an increased salivary lactobacilli count with probiotics application.²⁵ However, this issue has been not clearly demonstrated so care must be taken to prescribe the probiotic products for patients who are suffering untreated dental caries.²⁶

CONCLUSION

Kefir drink and sodium fluoride mouth rinse were equally effective in reducing the count of salivary mutans streptococci. Beside of good oral hygiene, consumption of Kefir drink is especially suggested for teenagers. Further studies with larger sample size and preferably, both on low- and high-risk population should be done to confirm the present results.

CLINICAL SIGNIFICANCE

Probiotics containing dairy products was known with anti-cariogenic properties. These agents may be used in caries control strategies adjunctively.

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