

## Evaluation of the effect of Kidi Lact probiotic product on streptococcus mutans in saliva in a group of 8-12-year-old children

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### Original Article

#### Abstract

**BACKGROUND AND AIM:** Previous studies have shown that lactobacillus presented in food products affects oral ecology. In this study, we decided to show the effect of periodic use of Kidi Lact, a product containing probiotic bacteria which is recommended for improvement of gastrointestinal (GI) function in babies and children by physicians, on level of streptococcus mutans (*S. mutans*) in saliva among children between 8-12 years old. There are no previous studies in this relation.

**METHODS:** This randomized crossover double-blind study was conducted on 30 healthy children between the age of 8-12 years. It included four steps. In the first step, children randomly were divided in two groups; in the step two, one of them used 1gr Kidi Lact every day and other group used placebo every day during two weeks, randomly. In the third step, children used none of them for one month, and finally in the step four, those who used Kidi Lact at step two, used placebo and other group vice versa. At the end of each step, samples of the children saliva was prepared and then, the number of *S. mutans* in the non-stimulated saliva samples was measured with microbial culture method. SPSS statistical software was used for data analysis.

**RESULTS:** *S. mutans* reduction in saliva after using Kidi Lact was statistically significant ( $P < 0.001$ ), but it showed no significant reduction after using placebo ( $P = 0.100$ ). The amount of *S. mutans* in the two groups had no significant difference before using Kidi Lact and placebo ( $P = 0.406$ ). Amount of *S. mutans* in the groups after using placebo and Kidi Lact was significant ( $P < 0.001$ ).

**CONCLUSION:** According to the present study, periodic use of Kidi Lact as a mouth wash has a strong effect on amount of *S. mutans* in saliva. So, physicians can be advised to tell their patients about rinsing of Kidi Lact before its swallowing.

**KEYWORDS:** Streptococcus Mutans; Saliva; Probiotic

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Currently, use of probiotic bacteria has been established for the prevention of some medical conditions such as gastrointestinal (GI) tract disorders and genitourinary system diseases. These useful bacteria which are mostly from the lactobacillus family occupy

the positions of harmful and pathogenic bacteria, resulting in a decrease in their counts, finally decreasing the incidence and severity of the condition.<sup>1</sup> In relation to the prevention of dental caries, as a condition whose etiologic factor is streptococcus mutans (*S. mutans*), with the use of products

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containing probiotic bacteria (such as ice cream and yoghurt), studies have yielded very favorable results. In this context, too, probiotic bacteria occupy the sites in the dental plaque that are usually occupied by *S. mutans*, resulting in a decrease in their counts in the dental plaque, finally decreasing the pathogenicity of *S. mutans* as an etiologic factor for dental caries. However, it should be noted that this positive effect depends on the duration of the use of probiotic products, and termination of the use of such products has again resulted in an increase in *S. mutans* counts in the dental plaque.<sup>2-4</sup>

Since dental caries is highly prevalent and preventive measures have not been able to completely overcome this problem in many countries and also since research on the use of probiotics to control dental caries has not been sufficient, we decided to evaluate the effect of one oral therapeutic product, containing probiotic bacteria on *S. mutans* counts in the dental plaque of a group of children.

The product is Kidi Lact (Zist Takhmir Co., Iran) and has been marketed in Iran for restoring normal flora and improving GI function in infants and children through its repeated oral use via dissolving in water. An interview to some of pediatricians showed this product as prescribed a lot by them. Based on the data provided by the manufacturer, this product contains high concentrations of 7 probiotic bacterial species, consisting of 6 lactobacillus species such as lactobacillus casei and one streptococcus species (*S. thermophilus*); these bacteria help re-establish the normal microbial flora of the GI tract, exerting various protective effects on the body. In addition, the manufacturer has reported that the product is effective in the treatment of different infectious diarrheas, traveler's diarrhea, antibiotic-induced diarrhea, chronic diarrhea, flatulence, and colitis, and helps prevent and resolve different infections by strengthening the immune system and exerting protective

effects on pathologic bacteria, with a great role in providing the factors necessary for children's growth. On the other hand, the manufacturer has reported a total colony-forming unit (CFU) of  $10^9$  for useful bacteria in the product, with other non-bacterial ingredients as follows: fructo-oligosaccharides (FOS) as a prebiotic agent, lactose, colloidal silicone dioxide, flavoring agents, and magnesium stearate. Therefore, the manufacturer has reported that Kidi Lact is a symbiotic product that contains both probiotic bacteria and prebiotic beneficial ingredients providing a proper environment for the survival and proliferation of probiotic bacteria. As a result, it has recommended that although the product can be stored at room temperature for 2-3 weeks, it is advisable to preserve it at 2-8 °C in a refrigerator to extend the survival of probiotic bacteria. The packaging of the product consists of 15 sachets in one pack and based on the physician's recommendations, it is possible to dissolve 1-2 sachets in water, fruit juice, milk, or the child's food for the child's consumption (the instructions pamphlet).

Previous studies have shown that lactobacillus presented in food products affects oral ecology. The aim of this study was to investigate the effect of periodic use of Kidi Lact, a product containing of probiotic bacteria which is recommended for treatment of GI disorders in babies and children by physicians, on level of *S. mutans* in saliva among children.

## Methods

The protocol of this randomized crossover double-blind study was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran, under the code of IR.MUMS.6d.REC.1394.265. (IRCT Code: 20161007030193N2). Thirty 8-12-year-old children from an institutional care center were included in this study on a voluntary basis. All the children and their guardians accepted to take part in the study and signed informed consent forms after

receiving the necessary explanations.

The inclusion criteria consisted of the following: thorough physical health, absence of any systemic disorder, no use of antibiotics during the 1-month period before the study, no use of fluoride-containing mouthwashes or gels, no use of xylitol-containing chewing gums during the 1-month period before the study, absence of untreated cavitated teeth, and a daily habit of tooth brushing.<sup>5</sup> Subjects with signs of dental caries, those lacking compliance, those taking antibiotics, those with systemic conditions, and those with any of the conditions discussed in the inclusion criteria section were excluded from the study.

The study design was double-blind and randomized crossover.

The procedural steps consisted of 4 stages as follows:

**Stage 1 (run-in):** The subjects were asked not to use any probiotic products for 1 week and observe routine oral hygiene measures. After 1 week, the authorities in the institutional care center asked the subjects to collect their unstimulated salivary samples at one specific hour in one minute in coded containers. Since unstimulated salivary samples are normally collected after at least one hour of abstinence from eating, drinking, chewing gums, and tooth brushing, the salivary samples were collected before breakfast. After this step, the subjects were assigned to two groups by the use of random numbers table, and the second stage of the study was initiated.<sup>5</sup>

**Stage 2:** The authorities in the center were asked to give a 1-gr sachet of Kidi Lact product dissolved in water to the subjects to gargle daily for two weeks and to give the second group, during the same period, a placebo solution.<sup>5,6</sup> The placebo solution contains all of Kidi Lact compounds except the bacteria. Both of them were similar in color and taste. During this stage, the researchers were blinded to group assignments in relation to the use of the probiotic product. At the end of this stage,

too, unstimulated salivary samples<sup>6</sup> of the subjects were collected in coded capped containers<sup>5</sup> and sent to the laboratory to determine *S. mutans* counts.

**Stage 3 (wash-out):** In this stage of the study, the subjects received their normal diets for a month and after this 1-month period, the subjects did not consume any probiotic products for 1 week and observed their routine diet based on the inclusion criteria. After this 1-week period, too, the subjects' unstimulated salivary samples were collected and sent to the laboratory to determine *S. mutans* counts.<sup>5-7</sup>

**Stage 4:** In this stage, the authorities in the center were asked to give a 1-gr sachet of Kidi Lact probiotic product or placebo daily. In this design, those taking placebo in the previous step received the probiotic product, and vice versa. After two weeks, unstimulated salivary samples of the subjects were collected and *S. mutans* counts were determined.

The laboratory techniques used in the present study to determine *S. mutans* counts included homogenization subsequent to culturing of salivary samples and using of gram-staining and various diagnostic tests to identify other bacterial species, which have been used in similar studies too.<sup>7,8</sup>

Data were not normal ( $P < 0.050$ ); so, Mann-Whitney U test was used to compare the number of *S. mutans* in saliva in both case and control groups. In addition, Wilcoxon test was used to measure the number of *S. mutans* before and after process.

## Results

*S. mutans* reduction in saliva after using Kidi Lact was statistically significant ( $P < 0.001$ ), but it showed no significant reduction after using placebo ( $P = 0.100$ ). The amount of *S. mutans* in the two groups had no significant difference before using Kidi Lact and placebo ( $P = 0.406$ ). Amount of *S. mutans* in the groups after using placebo and Kidi Lact was significant ( $P < 0.001$ ) (Tables 1 and 2).

**Table 1.** Salivary streptococcus mutans (*S. mutans*) counts in the four stages of the study after the use of placebo and Kidi Lact probiotic product

Groups	Before using the product (group 1)	Before using the product (group 2)	After using the product (group 1)	After using the product (group 2)
Placebo (CFU/ml)	$23.7 \times 10^6$	$42.3 \times 10^6$	$33.6 \times 10^6$	$35.9 \times 10^6$
Kidi Lact (CFU/ml)	$31.3 \times 10^6$	$37.6 \times 10^6$	$3.6 \times 10^6$	$2.0 \times 10^6$

CFU: Colony-forming unit

### Discussion

According to the present study, periodic use of Kidi Lact as a mouth wash has a strong effect on amount of *S. mutans* in saliva. One of the most novel methods to prevent and decrease the incidence of dental caries is bacteriotherapy through which the counts of harmful and cariogenic bacteria decrease in the oral cavity, resulting in a decrease in the incidence of dental caries. Use of probiotics is one of the bacteriotherapy techniques to improve the oral health status. Of all the useful probiotic species lactobacillus and bifidobacterium species are more predominantly used in probiotic and dairy products. These bacterial species decrease the salivary counts of *S. mutans* through different techniques such as competition for initial colonization and formation of bacterial colonies in the dental plaque, competition for nutrients in the dental plaque ecosystem, and a direct effect through bacteriocins. Therefore, based on the previous studies on the effect of probiotic products on orodental health, in the present study, the effect of a new probiotic product, Kidi Lact, on decreasing the salivary counts of *S. mutans* was evaluated.<sup>8-11</sup>

No previous study is available on the effect of Kidi Lact product on oral *S. mutans* counts; therefore, the results of the present study should be compared with those of

studies on products such as yoghurt, spar, and ice cream containing probiotic bacteria in their composition.

In a number of studies by Caglar et al.<sup>7,12,14-16</sup>, use of dairy products containing probiotic agents resulted in a significant decrease in the salivary counts of *S. mutans*. Since Kidi Lact product contains a large number of probiotic bacteria, the results of the present study are consistent with those of previous studies and it can be concluded that use of products containing probiotic bacteria results in a decrease in the salivary counts of cariogenic bacteria. Such a decrease in the counts of these bacteria results from competition of bacterial species for colonization in the oral cavity.<sup>14,17</sup>

Nase et al. evaluated the effect of long-term use of milk that contained probiotic bacteria of the lactobacillus rhamnosus species, which is present in the Kidi Lact product, on the salivary counts of *S. mutans* and in plaque samples in 1-6-year-old children. The results showed that the *S. mutans* counts and the overall rate of dental caries decreased, which is consistent with the results of the present study.<sup>18</sup>

Nikawa et al. reported a significant decrease in salivary counts of *S. mutans* after consuming probiotic yoghurt;<sup>19</sup> in the present study, too, a significant decrease was observed in the salivary counts of *S. mutans* after the use of Kidi Lact probiotic product.

**Table 2.** Comparison of mean of streptococcus mutans (*S. mutans*) counts between the two groups

Groups	Before using (mean ± SD)	After using (mean ± SD)	P
Placebo (CFU/ml)	$33.0 \times 10^6 \pm 1.59 \times 10^7$	$34.7 \times 10^6 \pm 1.24 \times 10^7$	0.100
Kidi Lact (CFU/ml)	$34.4 \times 10^6 \pm 1.13 \times 10^7$	$2.8 \times 10^6 \pm 6.46 \times 10^6$	< 0.001
P	0.406	< 0.001	-

SD: Standard deviation; CFU: Colony-forming unit

Poureslami et al.<sup>8</sup> carried out a study on fifty 13-17-year-old adolescents and reported that spar (an indigenous probiotic dairy product in Kerman Province, Iran) had a significant effect on decreasing salivary counts of *S. mutans*, consistent with the results of the present study. The procedures in that study were exactly similar to those in the present study; however, Poureslami et al.<sup>8</sup> included adolescents in their study, while the present study was carried out on children. Both studies were carried out in Kerman Province and the technique used to evaluate *S. mutans* counts (microbial culture) was exactly similar in both studies.

Lesan et al. evaluated the effect of the use of probiotic yoghurt on *S. mutans* and lactobacillus counts in unstimulated salivary samples of 32 subjects with *S. mutans* and 28 subjects with lactobacillus (with an age range of 23-55 years in both groups). The results showed that use of probiotic yoghurt resulted in a non-significant decrease in *S. mutans* counts; however, there was a significant decrease in salivary counts of

lactobacillus. The study above was carried out using a crossover design, similar to the present study. The researchers pointed out that non-significant decrease in *S. mutans* counts, contrary to the results of other studies, might be attributed to the study design, the age of the subjects, the duration of the use of probiotic products, the culture media used, and more importantly to the type of the probiotic product used, considering the strain-specific effect of probiotics.<sup>20</sup>

### Conclusion

According to the present study, periodic use of Kidi Lact as a mouth wash has a strong effect on amount of *S. mutans* in saliva. Therefore, physicians can recommend Kidi Lact to be rinsed before swallowing.

### Conflict of Interests

Authors have no conflict of interest.

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