Original Article

Effectiveness of probiotic, chlorhexidine and fluoride mouthwash against *Streptococcus mutans* – Randomized, single-blind, *in vivo* study

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Abstract

Aim: To determine the short-term efficiency of probiotic, chlorhexidine, and fluoride mouthwashes on plaque *Streptococcus mutans* level at four periodic intervals. **Materials and Methods:** This was a single-blind, randomized control study in which each subject was tested with only one mouthwash regimen. Fifty-two healthy qualified adult patients were selected randomly for the study and were divided into the following groups: group 1- 10 ml of distilled water, group 2- 10 ml of 0.2% chlorhexidine mouthwash, group 3- 10 ml of 500 ppm F/400 ml sodium fluoride mouthwash, and group 4- 10 ml of probiotic mouthwash. Plaque samples were collected from the buccal surface of premolars and molars in the maxillary quadrant. Sampling procedure was carried out by a single examiner after 7 days, 14 days, and 30 days, respectively, after the use of the mouthwash. All the samples were subjected to microbiological analysis and statistically analyzed with one-way analysis of variance (ANOVA) and *post-hoc* test. **Results:** One-way ANOVA comparison among groups 2, 3, and 4 showed no statistical significance, whereas group 1 showed statistically significant difference when compared with groups 2, 3, and 4 at 7th, 14th, and 30th day. **Conclusion:** Chlorhexidine, sodium fluoride, and probiotic mouthwashes reduce plaque *S. mutans* levels. Probiotic mouthwash is effective and equivalent to chlorhexidine and sodium fluoride mouthwashes. Thus, probiotic mouthwash can also be considered as an effective oral hygiene regimen.

Key words: *Chlorhexidine, probiotic mouthwash, sodium fluoride, Streptococcus mutans*

INTRODUCTION

In the present era, dental caries is the most common chronic oral disease that affects 60–90% of the young population. Dental caries has multifactorial etiology, and hence, dietary counseling and proper oral hygiene habits are required for its control.[1] Preventive dentistry in clinical practice has been evolving over a period of time in reducing the risk of caries in highly prone individuals. Early intervention and prevention of bacterial growth reduces its permanent colonization and prevents destruction of the tooth structure. *Streptococcus mutans*, the microbial species most strongly associated with carious lesion, is naturally present in the human oral plaque.[2] Mechanical plaque control by tooth brushing is the advisable and commonly practiced oral hygiene measure, although numerous anti-plaque agents have been in use as auxiliary aids. Using mouthwashes is an effective and safe method for delivery of antimicrobial agents and they are being used widely. These agents are capable of preventing bacterial adhesion, colonization, and metabolism, and thus affect the bacterial growth.[3] Lang *et al*. report

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that use of antimicrobial mouthwash for 30 s once a day as an adjunct to daily tooth brushing will reduce gingivitis and caries incidence within 6 months in children.[4] The mouthwashes as antimicrobial agents have a good potential in reducing the *S. mutans* level in saliva, but regular use of these agents can cause significant adverse effects like staining of teeth and drug resistance. To overcome the drawbacks of antimicrobial chemical agents, probiotic therapy can be considered as a viable alternative for oral care.[5] Probiotics are living microbes or ingredients containing living microbes that beneficially influence the health of the host. Several studies suggest that consumption of products containing probiotic lactobacilli or bifidobacteria could reduce the number of mutans streptococci in saliva.[6,7]

The objective of the current study was to analyze the short-term effectiveness of probiotic, chlorhexidine, and fluoride mouthwashes on plaque *S. mutans* level at four periodic intervals.

MATERIALS AND METHODS

The study was conducted according to the protocol approved by the Saveetha University Ethical Committee and Research Review Board and was funded by ICMR (Reference ID: STS 2013-01950). This was a single-blind, randomized study in which each subject was tested with only one regimen. Fifty-two healthy qualified adult volunteers of both genders and aged 18–25 years were selected for assessment. Inclusion criteria for the study were: Subjects with good dental and general health, not having used antibiotics or mouthwashes for 4 weeks before the start of the study, and following regular oral hygiene measures. Volunteers should have more than 1 × 104 colony forming units (CFU) of *S. mutans* to meet the inclusion criteria. Exclusion criteria were: Subjects with Oral hygiene index score >4, concurrent periodontitis, malaligned or overcrowded teeth, history of allergies, metabolic diseases such as diabetes or other medical conditions that could interfere with the study, drug addicts, pregnant or nursing women, and participation in any concurrent oral care study.

A written informed consent was obtained from all the volunteers before commencing the *in vivo* study. Oral prophylaxis was done for all the eligible subjects. All participants were taught to use modified bass technique for brushing. Volunteers were randomly divided into four groups with 13 patients in each group. Group 1 individuals were asked to rinse their mouth with 10 ml of placebo (distilled water), group 2 subjects used 10 ml of 0.2% chlorhexidine mouthwash (Hexidine mouthwash; ICPA, Mumbai, India), whereas group 3 subjects used 10 ml of 500 ppm F/400 ml sodium flouride mouthwash (Colgate Duraphat; Colgate-Palmolive, New York, USA) and group 4 subjects were asked to use 10 ml of probiotic mouthwash (DAROLAC; Aristo Pharmaceuticals, Chennai, India). All volunteers were instructed to rinse their mouth twice daily after brushing with non-fluoridated tooth paste (Dabur Meswak Tooth Paste; Dabur, Uttar Pradesh, India).

Prior to sampling, the subjects were asked to refrain from food and drinks for 90 min, except water. Six hours after the last brushing and rinsing, the participants were made to rinse with 10 ml of 10% sucrose for 2 min. Eight minutes after the sucrose challenge, a buccal plaque sample was collected from premolars and molars in the maxillary quadrant[4] with a Teflon spoon by a single experienced examiner. The sample collection procedure was performed 24 h after oral prophylaxis (baseline) and after 7 days, 14 days, and 30 days interval.

Microbiological analysis

The plaque samples were collected from the maxillary premolar and molar region using a Teflon spoon and transferred to sterile tubes containing 1.5 ml saline. Then, the samples were serially diluted in reduced transport fluid and 1 ml dilution was inoculated onto Mitis Salivarius agar plate and incubated at 37°C for 48 h. Colonies were counted with the help of a digital colony counter (Digital colony counter 363; Environmental & Scientific instruments Co, Haryana, India).

RESULTS

The mean CFU/ml of *S. mutans* was calculated at baseline (24 h) and after 7 days, 14 days, and 30 days of using mouthwash. The mean CFU/ml of all the study groups is given in Table 1 and Figure 1. A mean value

Table 1: Mean CFU/mL for all the groups

Groups	Baseline (24 hrs)	7 days	14 days	30 days
Group 1 (Distilled water)	1.9	17.95	20.25	70.33
Group 2 (0.2% CHX)	1.14	2.483	5.79	10.38
Group 3 (500 ppm F/ 400mL NaF)	1.2	5.224	7.52	19.76
Group 4 (Probiotic)	1.26	3.27	4.92	8.71

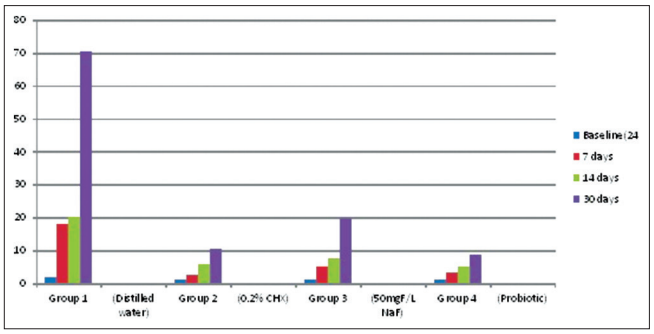

Figure 1: Mean CFU/ml for all experimental groups

of 1.9 was obtained at baseline, after which there was an increase in CFU to 17.95 on the 7th day. On the 14th day and 30th day, the mean CFU/ml was 20.25 and 70.33, respectively. Comparison within group 1 from baseline to 30th day showed maximum CFU increase from 1.9 to 70.33, which showed high statistical significance. For group 2 (chlorhexidine), the subjects showed a CFU/ml of 1.14 at baseline, which reduced to 2.483 on the 7th day and increased to 5.79 on the 14th day. On the 30th day, the mean CFU/ml showed a value of 10.38, and there was no statistically significant difference on comparing between the four intervals within the chlorhexidine group ($P > 0.05$). Group 3 (Fluoride) showed decrease in the mean CFU/ml, from 1.2 at baseline to 5.22 on the 7th day. The values on 14th and 30th day were 7.52 and 19.76 CFU/ml, respectively. No statistical significance difference was shown in group 3 at all the intervals. The mean CFU/ml of group 4 (probiotic) showed a value of 1.26 at baseline, which then increased to 3.27 CFU/ml and 4.92 CFU/ml on the 7th and 14th day, respectively. A value of 8.71 CFU/ml was obtained on the 30th day. The values at the four periodic intervals were not statistically significant.

One-way analysis of variance (ANOVA) comparison among groups 2, 3, and 4 showed no statistical significance ($P > 0.05$), whereas group 1 showed statistically significant difference when compared with groups 2, 3, and 4 on 7th, 14th, and 30th day ($P < 0.05$).

DISCUSSION

Maintaining good oral hygiene prevents most of the oral diseases. Oral hygiene habits among majority of the population include regular tooth brushing; but when mouthwash is used as an adjunct, it has positive synergistic effect in the oral cavity.[8] *S. mutans* is a gram-positive facultative anaerobe which initiates dental caries by metabolizing sucrose to lactic acid using the enzyme glucansucrase, creating an acidic environment in the oral cavity and thus facilitating demineralization of the enamel. It utilizes sucrose to form a dextran-based polysaccharide that helps in adhering to tooth surface, thereby forming dental plaque. Over 50 strains of *S. mutans* have been isolated, out of which 12 different strains are known to cause dental caries.[9]

Chlorhexidine gluconate is a cationic bisbiguanide having low toxicity and broad-spectrum antibacterial activity. When used as a mouthwash, it has a flushing action; its effects in the oral cavity are attributed to its lethal effects on the bacteria. It results in membrane disruption of the bacteria, causing a concentration-dependent growth inhibition and cell death. Secondary interactions leading to inhibition of proteolytic and glycosidic enzymes are also significant. The cationic nature of chlorhexidine helps it to bind to the tooth structure and oral mucosa, reducing pellicle formation and increasing substantivity through controlled release of the agent. It strongly inhibits plaque regrowth and prevents gingivitis.[4] Emilson CG (1981) studied the effect of chlorhexidine gel treatment on *S. mutans* population in human saliva and dental plaque, and concluded that using chlorhexidine for 14 days controls the oral infections caused by *S. mutans*.[10]

The chlorhexidine group (group 2) showed a decrease in the colony count on the 7th day due to the bactericidal effect of the mouthwash. On comparison with the baseline colony count of *S. mutans*, chlorhexidine mouthwash proved to be effective even on the 14th and 30th day with a reduced colony count, showing its substantivity. Huovienen *et al*. in his in vitro study, proves that *S. mutans* remained susceptible to chlorhexidine.[11] Jarvinen H *et al*. conducted an *in vivo* study for 1 week to check the efficacy of chlorhexidine on *S. mutans* and found an increased susceptibility of *S. mutans* to chlorhexidine in comparison with other antibacterial agents.[12]

The results of the present study show an increase in the colony count of 10.38 CFU on the 30th day in comparison with 1.14 CFU at baseline. The increase in bacterial count in 4 weeks period could be because of increased plaque accumulation or *S. mutans* could have developed drug resistance toward chlorhexidine. Milward and Wilson studied the effect of chlorhexidine on *Streptococcus sanguis* biofilm and concluded that 72-h biofilms tend to be more resistant to chlorhexidine than 24-h plaque biofilms.[13] But till date, *in vivo* resistance of *S. mutans* to chlorhexidine has not been documented in the literature.

Fluoride was introduced to dentistry by Sir H. Trendley Dean in the year 1934 due to its effect in

reducing demineralization of the tooth structure and increasing remineralization. Wide range of fluoride mouthwashes and tooth pastes are available to maintain oral hygiene and prevent caries.[14] Fluoride interacts with the metabolic and growth process in the bacteria by inhibiting the glycolytic enzyme which converts 2-P-glycerate to phosphoenol pyruvate (PEP). In the presence of fluoride, PEP inhibits the sugar transport in the PEP phosphotransfer system, causing bacterial cell death.[15]

In the present study, group 3 (fluoride mouthwash) showed a significant reduction in the colony count till the 14th day. In the 30th day sample, there was an increase in the colony count (19.76 CFU/ml), which could be due to drug resistance of *S. mutans*. Breaker studied the effects of fluoride on oral bacteria and hypothesized that fluoride riboswitch of *S. mutans* can push the fluoride ion from the cell membrane, thereby developing resistance.[16]

When the chlorhexidine mouthwash (group 2) was compared with the sodium fluoride mouthwash (group 3), even though it did not show a statistically significant difference, the reduction in the mean colony count in the chlorhexidine group was more than that of fluoride group on the 14th day. By the 30th day, both the mouthwashes showed bacterial resistance, but increased resistance was seen in sodium fluoride mouthwash group than the chlorhexidine mouthwash group based on the mean colony count. But continuous use of chlorhexidine mouthwash causes staining of tooth structure, irritation with burning sensation in the oral mucosa, and increased altered taste perception.[17] Thus, a probiotic mouthwash was prepared due to its minimal/no adverse effects in daily use along with tooth brushing.

Probiotic mouthwash contains living microbes, which beneficially influence the health of the host when used in adequate numbers. Consumption of products containing probiotic bacteria such as lactobacilli or *Bifidobacterium* could reduce the level of *S. mutans* in saliva. Lactobacilli are considered to be part of the resident oral microflora, but elevated counts have been found after treatment with probiotics. Probiotic bacteria are nowadays added to different commercial dairy products such as milk, cheese, yoghurt, chewing gums, fruit drinks, etc., They act by various mechanisms including competing for the binding site with the lethal bacteria, production of antimicrobial substances, and regulation of immune response. Bacterial antagonism occurs when the growth of one bacterium is hampered by another species.[18] Various studies have been conducted to analyze the association between caries-causing bacteria and use of probiotics. Hatakka *et al*. (2001) studied the effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and concluded that milk containing probiotic bacterium LGG had beneficial effect on children's dental health.[19] Keller and Twetman studied the acid production in dental plaque after exposure to probiotic bacteria and found no evidence of an increase in plaque acidity by the use of a probiotic.[20] Zahradnik RT *et al*. (2009) assessed the safety and effectiveness of a probiotic mouthwash and concluded that the product was safe for daily use as an aid in maintaining dental and periodontal health.[21] *Lactobacillus acidophilus* was used in our study because it can reduce the adherence of oral streptococcal strains to the tooth surface.[22]

The advantages of using probiotic strains are that the bacterial strains present in them are not harmful to the oral cavity, there is no issue of antibiotic resistance occurring, and there are no proven toxicities related to their use. In group 4 (probiotic mouth rinse), the mean colony counts were 1.26 CFU/ml at baseline, 3.27 CFU/ml on the 7th day, 4.92 CFU/ml on the 14th day, and 8.71 CFU/ml on the 30th day. There was a sustained decrease in the bacterial count even after the 14th day until 30th day of usage of the probiotic mouthwash.

When probiotic mouthwash (group 4) was compared with the chlorhexidine and sodium fluoride mouthwashes, the mean colony count of *S. mutans* was reduced on the 14th and 30th day. Among the three mouthwashes used, probiotic mouthwash samples gave the least CFU mean value. But there was no statistically significant difference among groups 2, 3, and 4. Thus, further studies have to be conducted in future to analyze the beneficial effects of probiotic mouthwash used for a longer duration in reducing oral *S. mutans* level.

CONCLUSION

According to the results of this *in vivo* study, chlorhexidine, sodium fluoride, and probiotic mouthwashes have statistically similar and equivalent antimicrobial effects on the susceptibility of oral plaque streptococcus mutans. Thus, probiotic mouthwash can also be considered as one of the effective regimens in maintaining oral hygiene.

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