Original article

To compare the effectiveness of different mouthrinses on Streptococcus mutans count in caries active children

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ABSTRACT

Introduction: Streptococcus mutans is the prime microbe responsible for caries. Mouthwashes represent an effective means for decreasing their count. To ascertain this, three commercially available mouthrinses were evaluated for their antimicrobial activity.

Materials and method: Chlorhexidine, Sodium fluoride and Herbal mouth rinse were evaluated for their efficacy against S. mutans in 60 children aged 6–12 years old. Plain water acted as the control group.

Results: The values obtained were subjected to statistical analysis. ANOVA test, student-t test and paired t-test were used for evaluation. Chlorhexidine and fluoride showed statistically significant reduction in S. mutans count as compared to herbal rinse.

Conclusion: All the mouthrinses used in the present study have shown a definite decline in S. mutans count.

1. Introduction

Dental caries is a ubiquitous disease that results from the interaction of specific bacteria and constituents of the diet within plaque formed on the tooth surfaces.¹ Streptococcus mutans are considered the main causative microorganism associated with dental caries.² A part of the caries control programs is the use of chemoprophylactic agents that target these S. mutans. Amongst the various antimicrobial delivery systems, mouthwashes have been found to be one of the safest and effective vehicles,² especially in young children, as they have the ability to deliver therapeutic ingredients to all accessible surfaces in the mouth including interproximal surfaces.³

Among the various mouthwashes the most persistent reduction of streptococcus mutans has been achieved by using chlorhexidine mouthwash.² Chlorhexidine is a cationic bisbiguanide, chemoprophylactic agent. Its mechanism of action is mainly based on the rupture of bacterial cell wall which subsequently causes precipitation of the cell cytoplasmic contents.⁴ In relation to Streptococcus mutans, Chlorhexidine displays its action by abolishing the activity of phosphoenolpyruvate and thus interfering with its metabolic activity.⁵

The major advantage of chlorhexidine over most other compounds lies in its substantivity. It binds to soft and hard tissues in the mouth, enabling it to act over a long period after use of a formulation.⁵ Addy and Wright found that bacterial counts in saliva consistently drop to between 10 and 20% of baseline after single rinse of CHX and remained at this level for next 7 h. However, Schiott et al in 1970 found its effect

to be more than 12 h.

Though it is used as a gold standard against which other antimicrobial agents are compared it has certain disadvantages like it alters the taste sensation (Addy et al, 1985), produces brown staining of teeth (Hoyos et al, 1977, Flotra et al) affects mucous membrane and tongue (Kenrad, 1990), sensitivity (Yusuf and Khoo, 1988) and has an unpleasant taste.^{2,6} Thus a long term use of chlorhexidine is not advocated.

Another mouthwash recognized as a potent anti-cariogenic agent commonly used in children is sodium fluoride mouthwash. Its pH is neutral and is available in concentration of 0.05% (220 ppm) for daily use and 0.2% (900 ppm) for weekly use. Fluoride displays its bacteriostatic properties by blocking an enzyme of the glycolytic pathway, enolase. Subsequently, inhibition of carbohydrate uptake by oral microbes via PEP (phosphoenolpyruvate) dependent phosphotransferase system (PEP-PTS) occurs.⁷ It alters the physicochemical properties of teeth by making them more resistant to acid dissolution due to the formation of fluorapatite. According to AAPD guidelines on fluoride therapy revised in the year 2014 they stated that 0.2% of NaF mouth rinse are effective in reducing dental caries in children.⁸ Though it has anticariogenic and remineralizing potential, yet accidental swallowing or prolonged use may lead to fluoride toxicity.

The commercially available antibacterial agents are reported over the centuries to have several side effects. Alcohol in mouthrinses, was considered as a pre-disposing factor in cancer of the mouth, but this was subsequently discounted (Smigel, 1991). Sodium retention in patients

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with hypertension, using Viadent, Plax, and Cepacol, was reported by Wagner et al (1989). Approximately 33% of the sodium contained in these mouthwashes was retained by the oral mucosa after rinsing.

The increase in the prevalence of side effects associated with antibacterial agents has encouraged the scientists to research for plant based antimicrobial agents. Patients are seeking products they perceive to be safer, healthier and without toxic chemical or synthetic ingredients.⁹

Various herbal mouthwashes are gaining popularity as they work without alcohol and colours and contain naturally occurring ingredients called phytochemicals that achieve the desired antimicrobial and anti-inflammatory effects. Hiora, a herbal mouthwash, contains the benefits of the following ingredients pilu, nagavalli, bibhitaki, yavani-satva, peppermint satva, gandhapura taila, ela. All these ingredients have been proven to individually possess high antimicrobial activity against *S. mutans*.^{10–14} The dental literature reveals the use of herbal mouthwashes mainly in adult population,^{3,15} not much research has been cited regarding its use in the pediatric population.

There is enough relevant evidence to suggest the antimicrobial activity of mouth rinses containing active ingredients (chlorhexidine, Hiora, fluoride) against streptococcus mutans. However comparison of the three for their antimicrobial efficacies in caries active children is sparse. Therefore, the purpose of the study was to evaluate the levels of *S. mutans* in children with high caries and to compare the efficacy of different mouthwashes in reducing the salivary *S. mutans* counts upto or comparable to caries free individuals.

2. Aim and objectives

The objective of this study was to evaluate the salivary *S. mutans* counts in caries active and caries free children and compare the effectiveness of Journal of Oral Biology and Craniofacial Research, doi: [10.1016/j.jobcr.2018.05.002](https://doi.org/10.1016/j.jobcr.2018.05.002) the commercially available mouth rinses i.e., chlorhexidine, Hiora [Himalaya] and sodium fluoride in reducing the salivary Streptococcus mutans count of caries active children.

3. Materials and method

The study was conducted in the Department of Pedodontics and Preventive Dentistry. All the necessary ethical guidelines were followed as prescribed by the Institutional Ethical Committee in accordance with the Central Ethics Committee on Human Research, India and ethical clearance was obtained.

The following mouthrinses were used in the study:-

0.2% of chlorhexidine gluconate (Hexidine^R), 200 ppm sodium fluoride mouth rinse (Fluoritop^R), herbal mouthwash from Himalaya company (Hiora^R)

4. Method

All patients in the age group of 6–12 years attending the OPD were screened for participation in the study. No treatment was performed on the patients during the time when the mouthrinses were used. The selection was made on the basis of certain criteria.

4.1. Criteria for selection

4.1.1. Inclusion criteria

- 1 Healthy children between ages of 6–12 years.
- 2 Patient having one or more than one interproximal carious lesions, well into the dentin, visualized radiographically.
- 3 All teeth and surrounding tissues were clinically and radiographically free of any other pathologic condition

4.1.2. Exclusion criteria

- 1 Physical limitations, which might preclude the normal tooth brushing and mouth rinsing.
- 2 Presence of any intraoral infections.
- 3 Medically compromised patients or those who were on any long term medication.
- 4 Subjects with history of taking antibiotics 1 month prior to or during the course of study.
- 5 Patient reporting with pain or history of pain requiring immediate treatment.

5. Clinical examination

Examination was done by a single examiner. The parents were informed about the child's condition. The purpose and method of study was explained to the children and parents/ Guardian. Written informed consent was obtained from parents/guardian for participation in the study.

5.1. Method of collection of saliva sample

All the children were instructed to have an early breakfast on the day of sample collection and there was a gap given of 90 min before the collection of saliva to avoid the influence of food consumption and contamination on the composition of saliva.¹⁶ The child was made to sit in an erect position on the dental chair and was given paraffin block to chew. Approximately 2–3 ml of the stimulated saliva was collected after 2 min of paraffin chewing in sterile containers held near the mouth. The containers were properly labeled on basis of the group which was allocated to them. The samples were stored at 4 °C till evaluation.

5.2. Distribution of samples

Group I (n = 15) – 10 ml of 0.2% chlorhexidine gluconate (Hexidine^R).

Group II (n = 15) 10 ml of herbal (Hiora^R) mouthwash.

Group III (n = 15) –10 ml of Sodium fluoride (Fluoritop^R) mouthwash.

All the mouthwashes were prescribed to be used twice daily in the interval of 12 h for 15 days to be used for full 1 min.

Group IV (n = 15) control group in which the children rinsed with plain water.

Salivary samples of 15 caries free children were also taken to estimate the baseline *S. mutans* counts.

The saliva samples for each child were collected on the first dental visit and again after 15 days of using the mouth rinse. They were then sent to the laboratory for microbiological assessment of Streptococcus mutans count.

5.2.1. The method of salivary sampling for estimating Streptococcus mutans

Mitis-Salivarius-Bacitracin agar (Gold et al, 1973) was used as the media for *S. mutans* culture.¹⁷ The plates were incubated within 4 h at 37 °C for 48 h under 5–20% CO₂. Streptococcus mutans colonies were identified as highly convex, round or spherical, raised, dark blue in color, ranging from a pinpoint to pin head size with a rough surface using digital colony counter as shown in. The results were expressed in CFU. All plates were processed and examined by the same investigator. Confirmation of mutans streptococci was performed under light microscope.

6. Statistical analysis

The Excel and SPSS (SPSS Inc, Chicago, Version 21.0) software packages were used for data entry and analysis. Level of significance “p” value was kept at 0.05. p > 0.05 not significant, p < 0.05

Table 1

Mean Pre and Post-rinse streptococcus mutans level comparison of the study participants in each study group +.

Study Groups	N	Mean DMFT	Mean Pre- rinse S. mutans count (CFU)	Mean Post rinse S. mutans count (CFU)	p-value
I	15	5.93 + 1.100	1.259 * 10 ⁵	0.214* 10 ⁵	0.003
II	15	5.20 + 1.521	0.649* 10 ⁵	0.297* 10 ⁵	0.057
III	15	6.87 + 1.885	1.624* 10 ⁵	0.238* 10 ⁵	0.028
IV	15	5.73 + 1.280	0.795* 10 ⁵	0.786* 10 ⁵	0.184
Total	60	5.93 + 1.561	1.082* 10 ⁵	0.384* 10 ⁵	0.001

+ Paired t-test applied.

significant. Mean values, ANOVA, student t-test and paired t-test were used for evaluating the data.

7. Results

The mean DMFT/deft scores in group I was 5.93 + 1.100, in group II was 5.20 + 1.521, in group III was 6.87 + 1.885 and in group IV was 5.73 + 1.280. The total mean DMFT/deft score was 5.93 + 1.561. The mean S. mutans count for the caries free subjects was 0.07400*10⁵ + 0.065119 with a DMFT/deft of zero. When the pre rinse and post rinse levels of S. mutans were evaluated, a decline in the S. mutans count was seen across all the groups as seen in Table 1. Group I had a pre- rinse value of 1.259*10⁵ and a post- rinse value of 0.214*10⁵ with a p- value of 0.003, Group II had a pre- rinse value of 0.649*10⁵ and a post- rinse value of 0.297*10⁵ but the result was not statistically significant with a p- value of 0.057, group III had a pre- rinse value of 1.624*10⁵ and a post- rinse value of 0.238*10⁵ and the result was statistically significant with a p- value of 0.028 while group IV had a pre- rinse value of 0.795*10⁵ and a post- rinse value of 0.786*10⁵ and the result was not statistically significant with a p- value of 0.184 as shown in Table 1.

The mean post treatment values of s.mutans counts of all the groups showed a non-significant p-value when compared with the caries free subjects as shown in Table 2.

8. Discussion

Dental caries is a multifactorial disease with several well-known components collectively acting for the disease to manifest. It is recognized to require a host (tooth), a dietary substrate and acidogenic bacteria.¹

Among pathogenic flora, Streptococcus mutans are considered the main causative microorganism associated with dental caries.² as smooth surface caries very rarely occurs in the absence of streptococci,¹⁸ though it can occur in the absence of lactobacilli. In the present study, high caries risk children were taken in accordance with AAPD guidelines for caries risk assessment.^{19, 20} The levels of S. mutans vary among the caries free and caries active subjects. The number of salivary S. mutans are found to be higher in caries active children. Correlation of S. mutans count with caries status of children has been carried out by numerous authors who have consistently found a higher number of S.

Table 2

Inter-group comparison of post-rinse values of all groups with CF subjects + +.

CF (MEAN S.MUTANS COUNT)	STUDY GROUP (MEAN S. MUTANS COUNT)	P-VALUE	N
0.07400* 10 ⁵	I (0.214* 10 ⁵ + 0.130)	1.00	15
0.07400* 10 ⁵	II (0.297* 10 ⁵ + 0.446)	1.00	15
0.07400* 10 ⁵	III (0.238* 10 ⁵ + 0.217)	1.00	15
0.07400* 10 ⁵	IV (0.786* 10 ⁵ + 1.405)	0.146	15

+ + Student-t test applied.

mutans in children having severe caries. (Kristofferson k et al (1986), Holbrook,²² Milgrom et al (2000), Hallonsten et al (1995), Matee et al, Cristiane Yumi Koga-Ito et al)

Douglass et al in 1996 performed a study to evaluate dental caries status and S. mutans levels in 4 year old Apache children. The results revealed that the mean dmfs of the children categorized in the high mutans streptococci range were greater than those of children categorized in the moderate range.²¹ 158 pre-school children aged 4 years were followed longitudinally for 2 years by Holbrook WP in 1993. Their mean dmfs was 3.3 at 4 years and it rose to 5.8 at 6 years.²²

In the present study a sample of 15 caries free children was evaluated for the presence of salivary S. mutans which was found to be 0.07400 × 10⁵. The total number of caries active children assessed were 60 where the mean DMFT/deft score was 5.93 and the mean S. mutans count was found to be 1.08 × 10⁵. These values are in accordance with the earlier studies which clearly establish that high DMFT/deft scores are associated with high levels of salivary S. mutans counts. Reduction of the microbial count is thus important to control the caries activity in individuals.

Since caries is an infection, it can be thought of being treated with antimicrobials. However, most of these agents are not selective and have a broad spectrum of action such as chlorhexidine, iodopovidine, fluoride, penicillin etc. It would be normal to find bacterial resistance to prolonged use of these agents. The use of chemotherapeutic agents was prescribed for 15 days as changes in the bacterial composition and bacterial population has been noticed after short term use of chemotherapeutic agents. Preventive strategies usually involve decreasing the growth or activity of S. mutans. There are laboratory and clinical evidence demonstrating that once the biofilm's bacterial ecosystem is free of S. mutans, this bacterium finds it difficult to be reintroduced due to competitive inhibition with other microorganisms. Thus reducing the s.mutans count might permit growth of other bacteria such as s. sanguinis and shift the acidic environment of the oral cavity towards a health promoting microbiota. (Andréa C.B. Silva et al, Ludovico Sbordone, Jens Kreth, 2005)

Larmas²³ and Messer²⁴ suggested that salivary mutans streptococcal tests can be used for pre-selection of patients for dental examination, demonstration of cariogenic infection, evaluation of the effectiveness of chemotherapeutic rinses and providing for an objective measure of treatment outcomes.

Thus, in the present study chlorhexidine, commercially available herbal mouth rinse, Hiora (Himalaya) and fluoride mouth rinse (NaF) were evaluated for their antimicrobial efficacy by estimating the salivary S. mutans counts pre-rinse and post-rinse for 15 days. The control group simply rinsed with plain water twice daily for 15 days.

8.1. GROUP I

In the present study 10 ml of 0.2% of chlorhexidine showed a significant reduction (p-value of 0.003) of s.mutans in 15 days that was comparable to the caries free subjects. Loe and Schiott first discussed the use of 0.2% chlorhexidine mouth rinse, 10 ml (20 mg) twice daily for 60 s and stated that it leads to inhibition of plaque regrowth and gingivitis. However with its 0.12% concentration a 15 ml dose is required which makes it equivalent to 20 mg.²⁵

Jain I and Jain P too found a marked downfall in S. mutans count from 4.72* 10⁵ + 0.363 to 1.109* 10⁵ + 0.394 after 2 weeks of rinse with chlorhexidine.²⁶

The efficacy of different concentration of Chlorhexidine against S. mutans was evaluated by Jayaprakash et al.²⁷ A 0.12% was found to be more efficacious than 0.2% and 0.06% chlorhexidine.

The metabolic activity of S. mutans biofilms was studied by Cardoso et al when treated with mouthwashes having different compositions and found 0.2% concentration of chlorhexidine to be better than 0.12% of chlorhexidine.³

The findings of the present study are in accordance with the

previous studies.

8.2. GROUP II

A reduction in *S. mutans* count was observed with Hiora though it was not statistically significant (p-value of 0.057).

The effectiveness of *Tachyspermum ammi* seeds activity against *Streptococcus mutans* was analyzed by Khan et al where they found that these seeds serve as a great potential source to be used as a therapeutic agent against dental caries.¹⁰ Similarly the antimicrobial activity of *amomum subulatum* and *elettaria cardamomum* against dental caries causing microorganisms was studied by Aneja KR and Joshi R in the year 2009 and found that these substances had a strong anti-ariogenic potential against *Streptococcus mutans*.¹¹

The antibiofilm formation activity of *Terminalia bellerica* plant extract against *streptococcus mutans* and *streptococcus sobrinus* was assessed by Yadav S and it was found to be a strong inhibitor of *Streptococcus mutans*.¹² Chaudhari LKD assessed the antimicrobial efficacy of 9 essential oils and found that cinnamon oil has the highest while Wintergreen oil, showed no antibacterial activity.¹³ Assessment of immediate antimicrobial effect of miswak (*Salvadora persica*) extract was studied by Bhat PK where they found that it had significant detrimental effect on the dental caries causing micro-organisms.¹⁴

In the present study also, the decrease in the *S. mutans* count has been observed on using Hiora for 15 days but the decline in the number was not statistically significant. The post treatment *s. mutans* value was comparable to the caries free subjects which could be due to the comparatively low pretreatment counts of *s. mutans* in the sample.

8.3. GROUP III

In Group III which was the fluoride mouth rinse group, a p-value of 0.028 was found.

Several studies have been conducted since a long time to find the anticaries and remineralization potential of Fluoride mouth rinse.

Yoshihara evaluated the long-term use of fluoride mouth rinse on the salivary levels of *mutans streptococci* and *lactobacilli* on children aged 7, 10, and 12 yrs and found that children with fluoride mouth rinse had lower levels of *mutans streptococci*.²⁸

The efficiency of 0.2% sodium fluoride mouth rinse in prevention of dental caries was assessed by Aminabadi according to the DMFT index. They found that following the use of 0.2% sodium fluoride mouthwash, mean DMFT got declined.²⁹

Ripa and Leske,³⁰ Heidmann et al³¹ and Kaneko (2006) assessed the benefits to the primary teeth from a school-based fluoride mouth rinsing program and found that after rinsing with fluoride mouth rinse there were declines seen in caries levels.

The results of the present study collaborate well with the previous researches and the final value of *s. mutans* is comparable to caries free children.

8.4. GROUP IV

The reduction of *S. mutans* levels on rinsing with plain water was non-significant (p value = 0.184) this indicates that rinsing alone with plain water did not prove to be beneficial in reducing the microbial load and the use of chemoprophylactic agent as an adjunct to mechanical cleansing is needed for maintaining proper oral health.

9. Inter group comparisons

The studies related to inter-group comparison of chlorhexidine and Hiora have been carried out in the past. Jaidka S et al when compared the efficacy of chlorhexidine, xylitol and herbal (Hiora) mouth rinses on 7–14 years old children found that maximum reduction was seen in herbal group (Hiora) followed by xylitol group and the least in

chlorhexidine group.³² The study was conducted on caries free subjects wherein the mouthwash was used for 30 days. In the present study, with the mean DMFT/deft scores of 5.93 and using the mouth rinses for 15 days proved Hiora to be inferior to both Hexidine and fluoritop.

Narayan A and Mendon C in the year 2012 conducted a study to compare the effect of 4 mouth rinses namely, Triphala, Hiora, Chlorhexidine and colgate plax on plaque formation and found colgate plax to be least effective. Prasad KARV et al in the year 2015 performed a study to analyze the anti-plaque effectiveness of Herbal (Hiora) and 0.2% Chlorhexidine gluconate mouth rinse and stated that both the mouthwashes had similar anti plaque activity.¹⁰ The safety, efficacy and antimicrobial properties of herbal (Hiora) mouth rinse were compared with chlorhexidine mouth rinse by Bhat N et al. They found that the maximum zones of inhibition was formed by Chlorhexidine followed by Hiora against *s. mutans*. The antimicrobial effectiveness of Chlorhexidine mouthwash (Hexidine), Herbal Mouthwash (Hiora) and Pomegranate-containing Mouthwash (Life-extension) was studied by Dabholkar CS against *Streptococcus mutans* (*S. mutans*). Chlorhexidine (0.12%) was proven to be the best followed by hiora and Pomegranate mouthwash.

The results of the present study are in accordance with the previous studies. Both chlorhexidine and Hiora have been found to be effective in reducing the *S. mutans* count. However, the difference for Hiora group was not significant with p-value 0.057. There was more significant reduction in salivary *S. mutans* count with use of chlorhexidine rinse.

When inter group comparisons were made between chlorhexidine and fluoride mouth rinse, it was found that the reduction in *S. mutans* count in both the groups was statistically significant.

Kulakarni and Damle (2003) assessed the effectiveness of sodium fluoride, chlorhexidine and triclosan mouth rinses for their antibacterial activity against *S. mutans* and found that chlorhexidine to be the best. Sundas and Rao carried out a study to evaluate the efficacy of sodium fluoride (0.05%) and chlorhexidine (0.2%) mouth rinses on plaque reduction and found the effectiveness of both to be statistically equal.² Further the efficacy of Fluoride, Chlorhexidine and Fluoride-chlorhexidine combined mouthwashes on Salivary *Streptococcus mutans* count was evaluated by Sajadi et al (2015) and they found that fluoride-chlorhexidine mouth rinse displayed significant reduction of *S. mutans* count in comparison with fluoride alone.

Similar results were observed in the present study and the efficacy of fluoride has been established in reducing the *s. mutans* count.

The effect of herbal and fluoride mouth rinses on salivary counts of *Streptococcus mutans* in 12–15 years old school children was studied by Somraj V (2017). Each child was asked to use mouth rinse daily for 1 year and found that both the mouth rinses were equally effective in caries prevention and can be recommended for use in school-based health education program to control dental caries.

10. Inter-group comparison of post-rinse subjects with caries free subjects

The post-rinse *S. mutans* levels of all the mouthwashes were comparable to the *S. mutans* level of caries free subjects as the mean *S. mutans* value for each group dropped down to the level of caries free subjects. When subjected to statistical evaluation the difference seen between post-rinse and caries free subjects was non-significant.

11. Conclusion

Hexidine and Fluoritop both have shown equal efficacy in reducing the *S. mutans* levels after rinsing twice daily for 15 days. The reduced levels are comparable to the bacterial levels in caries free subjects. Hiora has also shown promising results and it can be assumed that further reduction would occur if it was used for a longer period. Long term studies are indicated to see the effect of herbal mouthrinses in

caries active children.

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Conflict of interest

None.

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