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Comparison of Antibacterial Effect of Fluoride and Chlorhexidine on Two Cariogenic Bacteria: An in Vitro Study

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Abstract

Statement of problem: Dental plaque is the main source for dental caries and there is no proper vaccine that can affect dental plaques.
Objectives: Daily use of an efficient anti-plaque product can be very beneficial in plaque control and, thus, prevention of caries. This study aims to evaluate the antibacterial effects of four products of Chlorhexidine and Fluoride on two types of cariogenic bacteria.
Materials and Methods: In this in vitro study, the antibacterial effect of Chlorhexidine and Fluoride (gel and solution) against Streptococci Sanguis and Sobrinus was evaluated. Chlorhexidine gluconate 1% gel (Corosodyl, France), Chlorhexidine gluconate 2% solution (Consepsis, Ultradent, US), Sodium fluoride 0.2% solution (Oral-B, US) and Acidulated Phosphate Fluoride 1.23% gel (Denti-Care, Canada) were used. The disc diffusion method was used for testing bacterial sensitivity. The data were analyzed using paired t-test and Chi-square test.
Results: In comparison with the negative control, each of the four gels and solutions showed antibacterial effects but the effects were not statistically significant for fluoride solution (P=0.217). For S. Sobrinus, the mean diameter of inhibition zone around the discs coated with fluoride gel (F g), fluoride solution (F s), Chlorhexidine gel (CHX g) and Chlorhexidine solution (CHX s) were 19, 9, 21.5 and 27.5mm, respectively. For S. Sanguis, the mean diameter of inhibition zone around the discs coated with F g, F s, CHX g and CHX s were 17, 11, 17 and 25mm, respectively. CHX s had the most effect on both bacteria and F s had the least. CHX g and F g were less effective than CHX s, respectively.
Conclusion: The results demonstrated that 2% CHX s and 1.23% F g can be effective on inhibition of the growth of some of cariogenic bacteria. Therefore, these agents can be used in the prevention of Early Childhood Caries.

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Introduction

Early Childhood Caries (ECC) is a global problem affecting children, their families and dental therapists as restorative treatments are unsuccessful due to the recurrence of caries.

In addition, due to lack of cooperation among children, it is very difficult to manage compliance

during treatment, thereby treatments bear high costs on families. On the other hand, extraction of the affected teeth causes aesthetic, psychological and nutritional problems in children. Solutions offered for preventing the occurrence of such caries include (1) Teaching mothers not to repeatedly feed their children while they are asleep; clean the surface of child's teeth after breastfeeding and/or bottle feeding as well as not frequently feeding their children with sweet liquids and food. (2) In the case of occurrence of such caries, the trend of their progression can be slowed or stopped by topical fluoride therapy. Unfortunately, these two traditional approaches are not fully effective since children cannot tolerate the discontinuation or reduction of feeding intervals while asleep, hence mothers continue to nurse using bottles.

Repeated fluoride therapy by dentists is also not fully effective because it has been demonstrated that fluoride ion affects the intact or relatively intact enamel. Fluoride is not entirely effective on the teeth with destroyed enamel and severely defunct dentin filled with bacteria. The mechanism of anti-caries effect for fluoride primarily influences the hydroxyapatite of the enamel and converts it to fluoro-apatite or hydroxy-fluoro apatite that shows more resistance against organic acids secreted by cariogenic bacteria. In recent years, new approaches have been considered to control ECC such as topical application of antibacterial compounds on the surface of those teeth that are at risk of or affected by ECC [1-3].

Chlorhexidine is an antimicrobial substance used as a bactericide mouthwash or gel in the oral cavity and is prescribed more in periodontal related diseases [4]. However, its effect on cariogenic bacteria has rarely been considered and evaluated. It has been argued that fluoride can also have a bacteriostatic effect on the bacteria causing tooth caries in concentrations higher than that of a mouthwash. The aim of current study was to evaluate the antibacterial effects of Chlorhexidine and Fluoride (gel or solution) on two types of cariogenic bacteria.

Materials and Methods

In this in vitro study, the antibacterial effect of Chlorhexidine and Fluoride gels and solutions against Streptococci Sanguis and Sobronus (with organizational codes 1449 and 1601) was determined. Chlorhexidine gluconate 1% gel (Corosodyl, France), Chlorhexidine gluconate 2% solution (Consepsis, Ultradent, US), Acidulated Phosphate Fluoride 1.23% gel (Denti-Care, Canada) and Sodium fluoride 0.2% solution (Oral-B, US) were used. The bacteria were provided by the Iranian Research Organization for Science and Technology and prepared in lyophilized form. The lyophilized bacteria were resuscitated in the sterile condition of the laboratory through the following method: first, a scratch was created on the closed glass tube containing the bacterium and the tube was thoroughly sterilized using 70% alcohol containing gas. Then, the tube was broken from the scratch site and 0.3-0.4 ml of tauroglycolate (Himedia Co., India) was added to the dry content of the tube. After unifying the suspension, it was transferred to the solid culture medium of each bacterium provided by the Iranian Research Organization for Science and Technology. The culture media were incubated at 37ºC for 48 hours in order to allow bacterial growth.

The disc diffusion method was used for testing the bacterial sensitivity. This method has been used in many studies, [5-7] where Poureslami et al. have used in-vitro and in vivo testing of herbal extracts against the bacteria of dental plaques. After bacterial growth, a microbial suspension was prepared and transferred to the plates containing culture medium using a sterile swap. Then, the sterile paper discs with a diameter of 5 mm (Blank disc, PadtanTeb, Iran) were coated with the gels and solutions and subsequently placed on the surface of the plates containing culture medium in equal distances. Because the paper discs were coated with equal amounts of the gels and solutions, a pea-size amount of each gel or a drop of each solution was put on the lamella, the disc was put on the gel or solution, and another lamella was put on the first lamella. The two lamellas were pressed against each other for less than 10 seconds, so that all discs were covered with equal amounts of the gels and solutions. Then, the plates were incubated at 35ºC for 16-18 hours. After this period, the area of no growth around the discs and the diameter of the inhibition zones measured in millimeters were characterized as relative bacterial sensitivity to the gels. In the study, empty blank discs were used as negative controls.

Data were analyzed using paired t-test and Chi-squared test in SPSS 18. Statistical significance was defined at $P<0.05$.

Results

In comparison to the negative control (empty blank disc), all gels and solutions showed antibacterial effect. However, this effect was not statistically significant for fluoride solution in relation to S.Sobrinus (P=0.408) while it affected on S.Sanguis (Table 1).

CHX solution had the most effect on both bacteria and F solutions had the least. CHX gel and F gel were less effective than CHX solution (Table 2 and Figure 1).

Discussion

Daily use of an efficient anti-plaque product can be very beneficial in plaque control and thus caries prevention. Some groups of antimicrobial products have been studied thus far. The most important of

Table 1: The antibacterial effect of the gels and solutions in comparison to a negative control, empty blank disc, (P value).

Gels and Solutions	S. Sobrinus	S. Sanguis
Fluoride gel (Denti-care)	0.047	0.001
Fluoride solution (Oral-B)	0.408	0.025
CHX gel (Corsodyl)	0.029	0.001
CHX solution (Consepsis)	0.010	0.001

Table 2: Mean diameter of inhibition zone (SD) around the discs coated with the gels and solutions (mm).

Bacteria	Empty Disc	Fluoride gel	Fluoride solution	CHX gel	CHX solution
S. Sobrinus	0	19±3	9±2.5	21.5±2	27.5±3
S. Sanguis	0	17±2.5	11±2	17±2	25±3.5

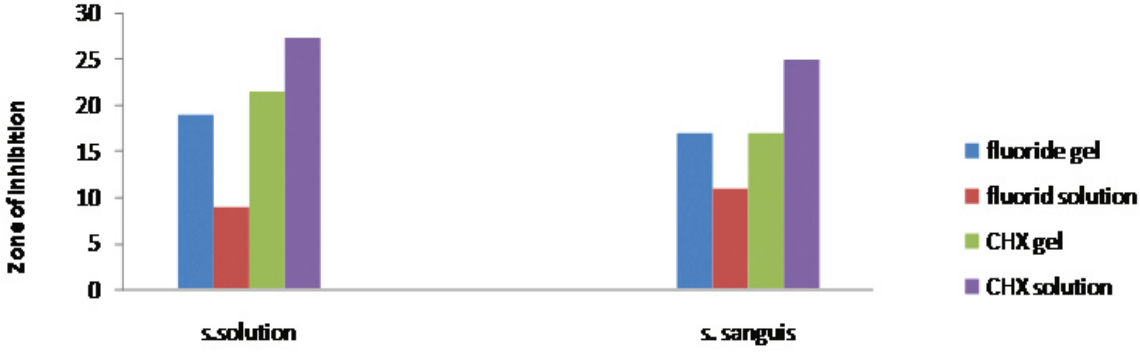

Figure 1: Mean diameter of inhibition zone around the discs coated with the gels and solutions (mm).

these compounds are positively charged organic molecules, halogens, metallic salts, and non-charged phenolic agents (herbal extracts). Each of these four groups has demonstrated positive results in clinical and laboratory studies [4].

In the present study, Streptococcus Mutans in lyophilized form could not be prepared and Streptococcus Sanguis and Sobronus were investigated. Chlorhexidine in both forms had more anti-bacterial effect than fluoride gel or solution. Chlorhexidine is a positively charged organic bactericidal agent. This agent binds with anionic glycoproteins and phosphoproteins on the tooth-borne pellicle. Its antibacterial effects include binding well to bacterial cell membranes, increasing their permeability, initiating leakage, and precipitating intracellular components [8].

However, fluoride is a Halogen agent and inhibits carbohydrate use of oral bacteria by blocking enzymes involved in the glycolytic pathway and acts as a bacterostatic agent. On the other hand, fluoride hampers the carbohydrate metabolism of the bacteria by incorporating fluoride as hydrofluoric acid and therefore causes cytoplasmatic acidification. This results in a non-specific inhibition of glycolysis because several enzymes including enolasse have their optimum efficacy in a neutral environment. Moreover the sugar transport system is sensitive to acidification of the cytoplasma. Both mechanisms reduce the generation of energy in the bacterial cell and production of lactate [9]; however, at preventive-use levels it probably does not alter the plaque ecosystem [4]. Increasing the fluoride ion concentration resulted in more bacteriostatic effect but its toxicity also increased [10].

Thus it is anticipatable which Chlorhexidine is more effective against the bacteria. Chlorhexidine solution showed more anti-bacterial effect than Chlorhexidine gel. The solution concentration is 2% but the gel concentration is 1%. Higher concentration is the most important factor for the efficacy of the solution. Chlorhexidine solution (2%) is used as an antiseptic agent in the canals in endodontic treatments routinely and Chlorhexidine gel 1% is prescribed for many preventive and/or therapeutic purposes [11,12]. Fluoride in the form of 1.23% gel had more anti-bacterial effect than the 0.2% fluoride solution. 1.23% fluoride gel is professionally used at dental clinics for prevention and controlling of dental decays. However, 0.2% fluoride solution is used weekly at home to increase enamel re-mineralization [4]. Therefore, as mentioned earlier, the fluoride gel that has more concentration of fluoride ion is more effective against bacteria.

There were no other studies similar to the current study in order to be included in the literature. A clinical study among disabled children showed 75% reduction in the plaque index after using Chlorhexidine spray

whereas in the Stannous fluoride spray group the reduction was 48% [13].

Children with ECC are three times more susceptible to carious lesions of permanent teeth in comparison with those without ECC, which influences oral health-related quality of life. Therefore, in order to decrease the rate of carious lesions in permanent teeth great efforts should be made to prevent the occurrence of ECC. In addition to instructing parents to prevent early contamination with *Streptococcus* in the oral cavity of breast-fed babies, efforts should be made to prevent the accumulation of these bacterial species at pathologic levels with the use of topical antibacterial agents [1,14].

Based on the some clinical studies [10,11] it has been recommended that fluoride should be used along with antibacterial agents, especially for children with limited access to health care services because topical fluoride does not provide a thorough protection against cariogenic bacteria. These studies [10,11] have recommended some antibacterial agents such as Chlorhexidine and povidone-iodine. Some studies have evaluated the effect of sodium fluoride (NaF) and povidone-iodine separately on salivary counts of Streptococcus, remineralization of white spots and ECC of deciduous teeth [10-12].

In a study by Lopeze et al., 12 to 19 month-old babies who had 4 intact incisors and were fed using milk bottle were divided into two groups [3]. In the test group, povidone-iodine was used every 2 months for 12 months. Placebo was used in the control group. The incidence of white spots was visually evaluated after a year. 91% of children in the test group were free of any carious lesions; however, only 54% of those in the control group were free of carious lesions [3].

Milgrom et al. evaluated the effect of a product of 5% NaF and 10% povidone-iodine on caries rate in deciduous teeth in 12-30 month-old children [14]. The children were divided into two groups. In one group only fluoride varnish was used and in the other group a product of the two materials mentioned above was used. It was reported that the product of the two materials decreased the rate of caries up to 13% more than the fluoride varnish alone. In the test group, in which the preparation was applied to the tooth surfaces 2-3 times during a 9-month period, only 41% of children developed new carious lesions; however, in the control group with the same applications of only fluoride varnish, 54% of children exhibited newly developed carious lesions [15]. Based on the mentioned studies, it is possible to combine fluoride with povidon-iodine. But it seems that it is not possible to combine fluoride ion with chlorhexidine because fluoride interferes with chlorhexidine components and reduces their antibacterial effects. These agents can be used separately but along with each other [11]. On the other hand, chlorhexidine is more suitable for this purpose because it has lower toxic effects on the oral cavity than Povidone-Iodine [16,17].

Conclusion

The results of this study demonstrated that Chlorhexidine solution 2% and Fluoride gel 1.23%, can be effective on inhibition of the growth of some cariogenic bacteria. Therefore, these agents can be used along with each other for synergic effects on the prevention and control of ECC.

Conflict of Interest: None declared.

References

1. Berkowitz RJ: Causes, treatment and prevention of early childhood caries. A Microbiologic perspective. J can Dent assoc. 2003;69:304-7.
2. Amin MS, Harrison RL, Benton TS, Roberts M, Weinstein P: Effect of povidone-iodine on Streptococcus mutans in children with extensive dental caries. Pediatr Dent. 2004;26:5-10.
3. Lopez L, Berkowitz R, Spiekerman C, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries: a follow-up report. Pediatr Dent.2002; 24: 204-6.
4. Mc Donald R, Avery DR, Dean JA. Dentistry for the child and adolescent. 9th Edition. St. Louis: Elsevier Science: USA 2011. P 181-215.
5. Poureslami HR, Makarem A, Mojab F. Para-clinical effects of Miswak herbal extract on dental plaque. Dent Res J. 2007;4:106-10.
6. Sanei AS, et al. Antimicrobial effects of seven plant extracts on pathogenic bacteria of the oral cavity. Pejouhandeh J. 1997;2:9-16.[In Persian]
7. Khordi-Mood M, Makarem A, Poureslami HR. An evaluation of the effect of a dentifrice containing herbal extracts on dental bacterial plaque. J Mashhad Dent School. 2002;26:47-56. [In Persian]
8. Pinar Erdem A, Sepet E, Kulekci G, Trosola SC, Guven Y. Effects of Two Fluoride Varnishes and One Fluoride/Chlorhexidine Varnish on Streptococcus mutans and Streptococcus sobrinus Biofilm Formation in Vitro. Int J Med Sci.2012; 9:129-136.
9. Campus G, et al. Fluoride concentration from dental sealants: a randomized clinical trial. J Dent Res. 2013;92:23S-8S.
10. Ohnmar K, Milgrom P, Tut M.Topical Iodine And Fluoride Varnish Combined Is More Effective Than Fluoride Varnish Alone For Protecting Erupting First Permanent Molars. A Retrospective Cohort Study. J Public Health Dent. 2010;70:249–52.
11. Amorim RG, Leal SC, Bezerra AC, de Amorim

FP, de Toledo OA. Association of chlorhexidine and fluoride for plaque control and white spot lesion re-mineralization in primary dentition. Int J Paediatr Dent. 2008;18:446-51.
12. Moraes S, et al. The Effect of 1% Chlorhexidine Varnish and 40% Xylitol Solution on Streptococcus Mutans and Plaque Accumulation in Children. J Pediatric Dent. 2011;33: 484-90.
13. Chikte UM, et al. Evaluation of stannous fluoride and chlorhexidine sprays on plaque in handicapped children. J Clin Periodontal. 1991;18:281-6.
14. Milgrom P, Kiet AL, et al. Xylitol pediatric topical oral syrup toprevent dental caries: a double blind randomized clinical trial of efficacy. Arch Pediatr Adolesc Med. 2009;163:601–7.
15. Milgrom P, Tut M, Ohnmar K.; Mancl, Lloyd A. Topical Iodine and Fluoride Varnish Effectiveness in the Primary Dentition: A Quasi-experimental Study. J Dent for Children.2011;78:143-7.
16. Smiratvir M, Shingh N, Chorpa S, Thomas AM. Efficacy of 10% povidone Iodine in children affected with early childhood caries : an in vivo study. J Clin Pediatr Dent.2010;34:233-8.
17. Zhan L, Featherstone JD, Gansky SA, Hoover CI, Fujino T, Berkowitz R, Den Besten PK. Antibacterial Treatment Needed for Severe Early Childhood Caries. J Public Health Dent.2006;66:174-9.