Original Study

THE EFFECT OF PASCAL AND PREVENTA FLUORIDE VARNISHES ON THE SALIVARY STREPTOCOCCUS MUTANS COUNT (IN VIVO)

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ABSTRACT

Aim: The aim of present study was to evaluate the effect of Pascal and Preventa fluoride varnishes on the salivary *Streptococcus mutans* count.

Materials & Method: A total of 38 children aged between 4 and 6 years with no caries or whose caries levels were moderate were enrolled into this study. A questionnaire was used to collect data concerning the child's oral hygiene routines, dietary habits and fluoride exposure. The children were randomly divided into two groups based on fluoride varnish: fluoride varnish by Pascal (n = 19) and fluoride varnish by Preventa (n = 19). After tooth brushing, additional unstimulated whole saliva samples were collected. Fluoride varnishes were applied for two groups. To determine the level of *Streptococcus mutans*, saliva samples were taken after 24h from all subjects, and cultured on a special laboratory media. Data were analyzed using independent-samples t-test and SPSS ver.22.

Results: The results showed that the level of bacterial growth, *Streptococcus mutans*, decreased in Preventa fluoride varnish group, but the difference was not statistically significant ($p>0.05$). The colony number of *Streptococcus mutans* decreased after using the Pascal fluoride varnish and the difference was statistically significant ($p<0.05$). Comparison between the two groups showed that there was no statistically significant difference between the two groups ($p>0.05$).

Conclusion: The results showed that both Pascal and Preventa fluoride varnishes were effective in reducing the number of salivary *Streptococcus mutans*.

Key words: Fluoride varnish, *Streptococcus mutans*, Saliva, Bacterial growth.

Introduction

The most prevalent oral infectious diseases are dental caries. Dental caries is the single most common chronic childhood disease.[1] The significant oral microorganisms involved in caries include *Streptococcus mutans*, Lactobacillus and some species of Actinomyces. *Streptococcus mutans* (*S. mutans*) are gram-positive bacteria which constitute biofilms on the surface of teeth. *Streptococcus mutans* are the super bug agent responsible for causing dental caries. The number of *mutans streptococci* in saliva can be used for the evaluation of caries risk and also for monitoring the level of microbial colonization. Prevention of dental caries can be achieved through maintaining oral hygiene by regular mechanical removal dental plaque. As a result, mechanical removal of dental biofilms is the preferred method for the prevention of Streptococci colonization.[2] Although mechanical plaque control acts as a source of support for prevention of oral diseases, chemical plaque control agents also act as useful adjuvants for maintaining oral hygiene.[3]

Fluorine is the most active chemical agent used due to its proved ability to prevent cavities. The mechanisms of fluoride include inhibition of demineralization, enhancement of remineralization, and inhibition of bacterial activity in dental plaque. This material is potentially bacteriostatic at lower concentrations and bacteriocidal at higher concentrations. The high concentrations of fluoride inhibit the irreversible solubilisation of tooth mineral by acid produced by certain bacteria that adhere to the tooth surface.[5] Fluoride may be in the form of a solution, gel, foam, or varnish.[6,7] Fluoride varnish is a highly concentrated form of fluoride which is applied to the tooth's surface as a type of topical fluoride treatment.[7] Fluoride varnish is particularly recommended for use in preschool-age children because of its ease of application and equal efficacy to fluoride gel system.[3] Fluoride varnish, due to its adherent nature is able to stay in contact with the tooth surface for several hours.[7] At the present time, there are more than 30 fluoride-containing varnish products on the market with different compositions. These compositional differences bring about broadly variable pharmacokinetics, the effects of which endure mostly untested clinically.[7]

Considering the Widespread use of fluoride and maximal beneficial effects of fluoride in caries prevention, the objective of present study was to compare the effect of fluoride varnish on salivary *S. mutans* count.

Materials & Method

The present study was a clinical trial study aimed at investigating the effect of Pascal and Preventa fluoride varnishes on salivary *S. mutans* count. A total of 38 kindergarten children aged four to six years old with no caries or whose caries levels were moderate were enrolled into this study. All children were in the primary or early mixed dentition and had no history of antibiotic use in the past month, as well as, the children did not have systemic prophylaxis for fluoride therapy. A questionnaire was used to collect data concerning the child's medical history, oral hygiene routines, dietary habits and fluoride exposure. Saliva samples were collected from 38 non-related volunteers after informed

consent obtained from the parents and approval from Ethics Committee of Ahvaz Jundishapur University of Medical Sciences. The children were randomly divided into two groups based on fluoride varnish: fluoride varnish by Pascal (n = 19) and fluoride varnish by Preventa (n = 19). After tooth brushing, additional unstimulated whole saliva samples were collected in random order by spitting into sterile tubes (walhin YB, 1991). After collecting saliva samples from the two groups, the Pascal fluoride varnish (Pascal, America) was applied for the first group and the second group were received the Preventa fluoride varnish (Asia Chemi Teb, Iran).

The teeth were first isolated from one another and dried using cotton roll prior to application of the varnishes. Then, a thin layer of fluoride varnish was applied to the teeth with a brush. One minute later, the cotton roll was removed from the children's mouth and then they were asked to expel their mouths and not to rinse, eat, drink or brush for at least 30 minutes. In order to equate behavioural performance, the children were asked to avoid brushing for 10 hours and also eating of hot, hard, and sticky foods were prohibited. After 24 hours, samples of saliva were again collected from both groups.

All of the data were recorded on the same day of the examination, all the salivary samples were transferred immediately to microbiological laboratory in sterile containers within one hour for microbiological analysis. *S. mutans* counts (cfu/mL) were obtained by direct counting on selective agar plates from saliva samples as follow: At the outset, dilutions of 0.01 and 0.001 was prepared from collected tube of saliva sample using physiological serum. Foremost, to prepare 0.01M of dilute solution, the saliva sample was vortexed and then 10 µl of saliva was added to 990 ml of physiological serum. Accordingly, 0.001M dilute solution was prepared by adding 100 µl of saliva to 1ml of physiological serum. Then, 10 µL of each dilute solution was sub cultured onto both Modified MSA (Himedia, India) and Blood Agar (Biolife, Italy) plates. The MSA medium plates were placed in under microaerophilic conditions (37°C for 48 hours) using Oxoid Campylobacter Gas Generating Kit (BR0056) in conjunction with the Oxoid Anaerobic Jar.

After incubation, all colonies on all plates were counted. As well as, the number of plates in first dilution was counted and the number of plates in second dilution was counted, accordingly. The morphology of the bacteria was then examined. The Cocci bacteria was observed in pairs (as diplococci), in groups of four (as tetrads), in chains (as streptococci), and in clusters (as staphylococci). The colonies of the developed bacteria were isolated and identified by using streptococcal diagnostic tests including catalase test, mannitol fermentation test, sorbitol fermentation test, Inulin, raffinose, and sucrose test, arginine dihydrolase and bile-esculin hydrolysis test. To determine the number of colony-forming unit (CFU) the colony number was multiplied by the dilution factor. Data were analysed using independent-samples t-test and SPSS version 22 $p< 0.05$ was considered significant.

Results

There was a significant decrease in biofilm production of *Streptococcus mutans* following Preventa varnish application, however, the difference was not statistically significant ($p > 0.05$). [Figure 1]

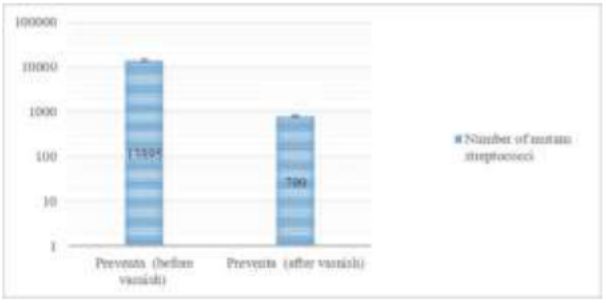

Figure 1. The mean number of salivary S. mutans value before and after Preventa fluoride varnish application.

The mean number of salivary *Streptococcus mutans* before Pascal varnish application was higher than the number of salivary *Streptococcus mutans* after Pascal varnish, which was statistically significant ($p < 0.05$). [Figure 2]

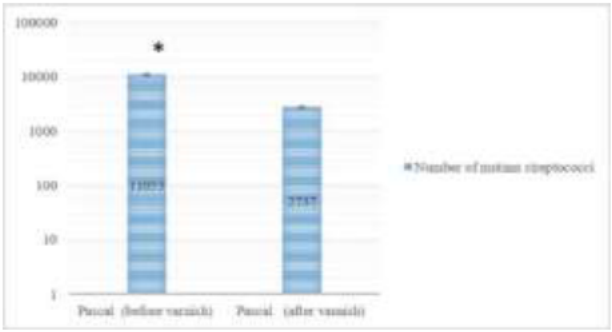

Figure 2. The mean number of salivary S. mutans value before and after Pascal fluoride varnish application.

According to Figure 3, the mean number of salivary *S. mutans* before the Preventa varnish application was greater than Pascal varnish, while the mean number of *S. mutans* after Pascal varnish was higher than Preventa varnish, but the difference was not statistically significant ($p > 0.05$). [Figure 3]

Figure 3. The mean number of salivary Streptococcus mutans value before and after the Preventa and Pascal fluoride varnish application.

Discussion

Tooth decay is a contagious disease which may become chronic following infection with decay-causing bacteria.[6,8] *Streptococcus mutans* is the most frequently isolated species and the primary etiological pathogen associated with the formation of dental caries.[4] Factors such as bacterial counts of *mutans streptococci* (MS) are systematic methods of caries detection, classification, and risk assessment, as well as can be used in prevention/risk management strategies and can help to reduce patient risk of developing advanced disease.[5]

Fluoride is known to be the most effective anti-caries agent in dentistry, especially in paediatric dentistry, and has changed from a therapeutic concept to a preventive approach in the last few decades.[5] Fluoride varnish is a topical fluoride that is recommended for preschool children, children with special care needs, and decalcified enamel related to nutritional disturbance or poor plate removal. The fluoride varnish hardens on the tooth the moment it contacts saliva, allowing the high concentration of fluoride to be in contact with tooth enamel.[6, 9]

Considering the medicinal use of fluorides for the prevention of dental caries the aim of present study was to compare the effect of Pascal and Preventa fluoride varnishes on salivary *S. mutans* count.

The present study showed a statistically significant reduction in salivary *S. mutans* counts following fluoride varnish application, but there was no statistically significant difference between the two groups.

The result of present study is consistent with the result of Deepti *et al*. study which indicated that the Fluor protector varnish is effective on the reduction of salivary *Streptococcus mutans* in saliva of caries free children.[5]

Erdem *et al*., 2012 in a study showed that all varnishes effectively inhibit the biofilm formation by *Streptococcus mutans* and Streptococcus sabrinus and concluded that adding fluoride to chlorhexidine mouthwash can significantly decrease salivary *S. mutans* count compared to fluoride alone.[4] The result of this study is consistent with the result of present study.

Mortazavi *et al*.,2007, in an in vitro study and in agreement with the present study, stated that sodium fluoride varnish reduce *Streptococcus mutans* adhesion level.[10]

Ekenback *et al*., 2000 in a study suggested that application of fluoride varnishes (Fluor Protector and Duraphat) express no greater statistically significant reduction of *Streptococcus mutans* after a week, a month, and six months. Inconsistencies between this result and the result of present study may be attributed to the age of the patients, materials and methods, and the sampling interval prior using varnish. In this study, samples were taken from a dental plaque, but in the present study the samples were taken from saliva.[11]

In general, the results specified that the application of fluoride varnish is effective in reducing the *Streptococcus mutans* count (CFU/ml of saliva).[1,2,4,6,8]

Conclusion

Regularly topical fluoride exposure can contribute to the remineralization of the smooth-surface decay and suppress the number of *mutans streptococcus* in the saliva. It is recommended that fluoride varnish rub on the teeth of all infants and children at least once every 6 months and preferably every 3 months.

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