

Comparing the Effect of Three Different Fluoride Varnishes on Salivary Fluoride Ions and Streptococcus Mutants' Levels: Clinical Trial

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ABSTRACT

Background: Fluoride content in saliva and dental plaque plays a key role in the prevention and control of dental caries. Fluoride varnish adheres to the tooth surface for longer period and prevents its immediate loss, thus acting as slow-releasing reservoirs.

Aim: to evaluate the effect of different fluoride varnishes forms on the level of fluoride ions in saliva and their antibacterial action on streptococcus mutans.

Materials and methods: Seventy five school children of age 12-15 years were selected fulfilling inclusion and exclusion criteria and divided into three groups: Group I (Duraphat varnish); Group II (Bifluorid 10) ; Group III (MI varnish). Salivary samples were collected and fluoride ions were recorded at baseline, one, two, 24,48 hours after applications. Salivary *streptococcus mutans* (CFUs X 10⁶) was assessed at baseline and after one, two, three, and four weeks of applications.

Results: Statistical significant higher mean values of fluoride ions Group II (Bifluorid 10); Group III (MI varnish) after one, two, 24, 48 hours of applications. Statistical significant reduction was detected in the streptococcus mutans level with the three used varnishes with different levels.

Conclusion: After 48hrs of fluoride varnishes application, (MI varnish) fluoride varnish with CPP-ACP was superior to other varnishes to maintain high level of salivary fluoride ions. Fluoride varnish with CPP-ACP and Bifluorid 10 varnishes keep the saliva with low level of cariogenic streptococcus mutans after four weeks of application.

Keywords: salivary fluoride ions, streptococcus mutans, fluoride varnishes, MI varnish, Bifluorid varnish

INTRODUCTION

The prevention of dental caries in children and adolescents is generally regarded as a priority for dental services and considered more cost-effective than its treatment. ^[1]

Fluoride found to be the most effective cariostatic agent in dentistry; the actions of fluoride for caries prevention are related to its effect on cariogenic bacteria, altering the salivary and plaque contents. ^[2]

Fluoride reducing demineralization and improving remineralization of the tooth structure, it can provide antimicrobial effect

by reducing bacterial metabolism and interfering in protons extrusion. ^[3] Studies have shown that high concentrations of fluoride are effective in reducing acid production and acid tolerance as well as extracellular polysaccharide formation of *Streptococcus mutans* biofilm. ^[4,5] Recently, Pandit et al., ^[6] showed that one minute of application of more than or equal 300 ppm fluoride was able to control cariogenic biofilm through inhibition of virulence properties. Fluoride can induce remineralization even with low concentration (0.02 ppm) in saliva and

plaque fluid, on the other hand, fluoride concentration below (10 ppm) cannot affect bacterial metabolism.^[7]

Varnish is the most best fluoride vehicle, it has the advantage of having resinous base, which allows a long contact time of the preventive agents with the tooth surface.^[8] Varnishes adhere to the tooth surface for longer period and prevent immediate loss of fluorides, thus acting as slow-releasing reservoirs.^[9]

Addition of calcium to the fluoride products especially varnishes have a promising results on caries prevalence, a novel material CPP-ACP is a product derived from milk, which strengthens and remineralize the tooth structure and has anticariogenic properties.^[10]

The present study was conducted to compare the effect of three novel fluoride varnishes on salivary fluoride ions level and streptococcus mutans level.

MATERIALS AND METHODS

A clinical trial was designed to assess the effect of three commercial fluoride varnishes (Duraphat, Bifluorid 10 and varnish containing CPP-ACP with fluoride (MI Varnish) on the salivary fluoride concentration and effect *streptococcus mutans* level. Ethical approval from Institutional Review Board, College of Dentistry, and Umm AlQura University (IRB no. 125 19) was obtained prior to the study.

Subjects

School children aged (12 – 15) years from of both genders were recruited and examined regarding the inclusion criteria. Children with no active carious lesions according to WHO criteria (0) with no history of antibiotic at least one month and no history of professionally applied fluorides at least six months were included in the study. Subjects not brushing their teeth or had severed gingivitis were excluded. From (278) subjects screened for the inclusion criteria (75) subject were selected and informed consents were obtained from them and their parents.

Finally (75) subjects were randomly distributed into three groups. Group I, II and group III (25) subjects in each one were the Duraphat, (Duraphat®, Colgate Palmolive GmbH, Hamburg, Germany) Bifluorid 10 (Bifluorid 10® VOCO GmbH Cuxhaven Germany) and varnish containing CPP-ACP with fluoride (MI Varnish® GC India Dental Pvt Ltd., India) were applied respectively.

Sample size and calculation:

Sample size was calculated using <https://clincalc.com/stats/samplesize.aspx> sample size calculator with confidence interval 95%, confidence level 5% and sample power 80%. The calculated sample size was (22) subject in each group with a total size (66) subject which was increased to avoid withdrawal.

Salivary sample collection

Two days before salivary sample collection, the subjects were abstained from any oral hygiene measures. At the collection day, the subjects were asked to refrain from eating or drinking two hours prior to collection of saliva. The saliva was collected between 8 am to 10 am. Collection of sample was carried out by suction method using sterile disposable syringes. Three ml of unstimulated saliva were collected and stored in ice container to send for laboratory investigations.

Varnishes application

Varnish application was carried out for each subject in the respective groups using paint on brush technique. Teeth were dried by the three way syringe and isolated using cotton rolls and saliva ejector. Approximately 0.1 ml of varnish was applied to all the teeth of subjects and allowed to dry for 30 seconds. Subjects were instructed not to rinse their mouth, not to eat/drink for three hours and not to brush till the next morning.

Fluoride ions analysis

Salivary samples were collected for each subject in the three groups five times for analysis the fluoride level (at baseline, after one, two, 24 and 48 hours). One ml of unstimulated saliva was taken and the

salivary fluoride content was analyzed using a fluoride-sensitive electrode (96-09 Orion, Thermo Electron, Beverly, MA, USA). All measurements were repeated three times and the mean of the measurements was calculated and used for further statistical evaluation.

Streptococcus Mutans analysis

One ml of the unstimulated salivary sample was collected at baseline and after one week, two, three and four weeks. Salivary samples were serially diluted using sterile 0.05 M phosphate buffered saline before plating. Culture media used were Mitis salivarius agar (MSA) (Acumedia, Baltimore, Maryland, USA) supplemented with filtrated 1% potassium tellurite (Difco Laboratories, Detroit, Michigan, USA) for isolation of *Streptococcus mutans*.

Statistical analysis

All data were analyzed using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA test was used to compare between the different fluoride groups at various time intervals. Bonferroni multiple comparison test for inter group comparison of *Streptococcus mutans* colony count. Intra group comparison at various time points was made by using paired t-test.

RESULTS

Fluoride levels in saliva after 48hrs of varnishes application were significant higher in the three groups compared with the baseline ($p = 0.000$). Higher means of fluoride ions among MI varnish group were observed during all observations periods with significance differences compared with Duraphat or Bifluorid groups. After 48hrs the mean fluoride ions in saliva among MI varnish group (2.909) was higher than the

other two groups (0.739 and 0.996 respectively) (table 1).

Table (2), showing the pairwise comparison between the three varnishes groups at the evaluation periods 1hr, 2hrs, 24hrs and 48hrs, Duraphate and Bifluorid groups showed no statistical significant difference in the mean fluoride level in saliva after 24hrs and 48hrs of applications ($p = 0.07, 0.09$ respectively). On the other hand, MI varnish showed higher significance differences in the mean fluoride levels compared to Duraphate and Bifluorid groups in the four evaluation periods.

Table (3), showed the *streptococcus mutans* level in saliva before application of different fluoride varnishes and after one, two, three and four weeks, the mean CFUs was decreased after one week of different fluoride varnishes applications, with significant differences between the baseline values and after one week values ($p = 0.000$). MI varnish group showed the lowest means of CFUs after one, two, three and four weeks of evaluations (4.911, 9.285, 17.809 and 43.431 respectively). Significant differences were found among the MI varnish group and Duraphate or Bifluorid regarding the four points of evaluations ($p = 0.02, 0.000, 0.01$ and 0.02 respectively).

Regarding the pairwise comparison between the three varnishes groups, Duraphate varnish group showed significant differences at one, two, three and four weeks with both Bifluorid and MI varnish groups. On the other hand, no significant differences were found between Bifluorid group and MI varnish group regarding the four points of evaluations ($p = 0.09, 0.07, 0.197, 0.07$ respectively) (table 4)

Table 1: The mean fluoride ions level in saliva at baseline and after one, two, 24 and 48 hours of application of three fluoride varnishes

	Duraphat	Bifluorid 10	MI varnish	F (p value)
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
Baseline	0.197 \pm 0.098	0.207 \pm 0.106	0.219 \pm 0.069	2.633 (0.973)
One hour	23.911 \pm 3.217	39.793 \pm 4.281	51.847 \pm 3.771	8.943 (0.01)*
Two hours	7.120 \pm 1.941	12.188 \pm 1.886	17.874 \pm 3.985	23.74 (0.000)*
24 hours	3.219 \pm 1.205	3.994 \pm 1.098	5.285 \pm 2.754	5.709 (0.01)*
48 hours	0.739 \pm 0.979	0.996 \pm 0.995	1.909 \pm 1.894	4.991 (0.01)*
F (p value)	44.243 (0.000)	38.611(0.000)	49.318 (0.000)	

F: ANOVA tests

*: significant at p value less than or equal 0.05

Table 2. Pairwise comparison using Bonferroni test between fluoride varnishes at different measurement periods

	Duraphat vs Bifluorid	Duraphat vs MI varnish	Bifluorid vs MI varnish
One hour	0.05*	0.001*	0.000*
Two hours	0.001*	0.001*	0.000*
24 hours	0.07	0.01*	0.01*
48 hours	0.09	0.05*	0.01*

*: significant at p value less than or equal 0.05

Table 3: The mean streptococcus mutans level (CFUs X 10⁶) level in saliva at baseline and after one, two, three and four weeks of application of three fluoride varnishes

	Duraphate Mean ± SD	Bifluorid 10 Mean ± SD	MI varnish Mean ± SD	F (p value)
Baseline	73.502 ± 6.805	69.097 ± 7.939	74.714 ± 4.760	1.957 (0.832)
One weeks	7.925 ± 3.322	5.116 ± 1.094	4.911 ± 1.031	5.392 (0.02)*
Two weeks	15.283 ± 5.365	9.775 ± 3.088	9.285 ± 1.119	27.436 (0.000)*
Three weeks	43.477 ± 8.473	18.011 ± 4.109	17.809 ± 4.216	8.969 (0.01)*
Four weeks	62.181 ± 11.612	49.749 ± 9.329	43.341 ± 8.994	5.493 (0.02)*

F: ANOVA tests

*: significant at p value less than or equal 0.05

Table 4. Pairwise comparison using Bonferroni test between fluoride varnishes at different measurement periods.

	Duraphat vs Bifluorid	Duraphat vs MI varnish	Bifluorid vs MI varnish
One weeks	0.05*	0.01*	0.09
Two weeks	0.001*	0.001*	0.07
Three weeks	0.001*	0.001*	0.197
Four weeks	0.05*	0.01*	0.07

*: significant at p value less than or equal 0.05

DISCUSSION

The present study was conducted to evaluate the effect of fluoride varnishes with different concentrations on fluoride content in saliva, the duration of effect and their relation to *streptococcus mutans* level in saliva. Subjects in the present study were selected from age 12 -15 years the most common age for development of dental caries. Standardization of the salivary sample collection time in the morning was used in order to avoid any bias, the use of unstimulated salivary sample to avoid any contamination and allow accurate estimation of fluoride ions level. [11]

The results of the present study demonstrated a peak increase of salivary fluoride concentration after one hour of varnish application and lasting for at least two hours. This increase still evident high than the baseline level even after 24 and 48hrs of application. The three formulations, (Duraphat, Bifluorid 10 and MI varnish) demonstrated the same trend, whereas the fluoride concentration after MI varnish administration was slightly higher. Results reported by Poursalami H et.al [12] showed that the salivary fluoride concentration was significantly higher in the

CPP-ACPF group compared to the other fluoride group without CPP-ACP.

The intraoral F retention or substantively depends upon different factors such as salivary flow rate, age, stimulation effects, properties of fluoride containing products, volume and application time, vehicle of fluoride delivery, individual characteristics of saliva, and post brushing rinsing behavior. [13] In the present study, the concentration of F in the dentifrice greatly influenced F retention in plaque, conforming to published findings by Duckworth et.al. [14]

While initial fluoride concentrations were higher in saliva than those obtained in plaque, it is important to consider that this increase has low substantiality with time, due to continuous self-cleaning with saliva and other fluids. Plaque, acting as a fluoride reservoir, maintained a constant F level. Zero et.al., [15] established that mean whole plaque F clearance curves followed roughly similar profiles to corresponding saliva F curves up to 2 h after F application, though the larger variations observed between subjects for the plaque data.

Fluoride also affects bacterial metabolism particularly acid production.

The present study was evaluated antibacterial effects of the fluoride varnishes, it was seen that, Bifluorid 10varnish have the highest inhibitory than Duraphate varnish, this might be attributed to the greater fluoride content of Bifluorid10 compared to low fluoride content of Duraphate. [16] A Study conducted by Zikert and Emilson [17] found that Duraphat varnish did not significantly affect the levels of *Streptococcus mutans* in saliva and dental plaque from children receiving varnish treatment.

Varnish containing fluoride with CPP-ACP was found to be effective in comparison to the other fluoride varnish groups; this could be due to the additive anticariogenic effect of CPP-ACP. Fluoride attributable to localization of ACPF at the tooth surface by the CPP which would co-localize calcium, phosphate and fluoride thus forming a reservoir and for slow and prolong release of ions for a longer period of time as seen in the present study.

The functional potential of CPP-ACP in inhibiting demineralization, increasing remineralization, and decreasing adhesion of *streptococcus mutans* and its bacteriostatic/bactericidal activity is similar to those of fluoride; however, it does not exhibit the detrimental effects of the excessive use of fluoride. [10, 18-20]

Duraisamy V et al., [21] stated that fluoride varnish with CPP-ACP was superior to fluoride or CPP-ACP applied alone. CPP also has shown to decrease the count of *S. mutans* as it has the ability to integrate in the salivary pellicle thus inhibiting its adherence. This is in accordance with the present study, where similar results evidence the synergistic effect of fluoride varnish with CPP-ACP.

The presence of fluoride ions in the oral cavity at the time when the pH is decreasing and the carious lesion is starting, inhibits demineralization of enamel by promoting remineralization. [22] The presence of low but constant concentration of fluoride in saliva and plaque fluid is the

most effective method to control initial dental caries.

CONCLUSION

Fluoride varnish with CPP-ACP could be considered effective in maintaining higher intraoral fluoride levels, and low level of salivary cariogenic bacteria in comparison to conventional fluoride varnishes. Therefore, fluoride varnish with CPP-ACP is recommended for high-risk patients as a cost-effective method of caries prevention.

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