

CLINICAL EVALUATION OF THE ANTI-BACTERIAL EFFECT OF FLUORIDE AND CHLORHEXIDINE VARNISHES

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ABSTRACT

Dental caries is one of the most prevalent infectious diseases and although of multifactorial origin, *Streptococcus mutans* is considered the chief pathogen in its development. Fluoride is one of the most effective agents used for the reduction of dental caries apart from oral hygiene maintenance. Chlorhexidine is the most potent documented antimicrobial agent against *Mutans streptococci* and dental caries.

Aims: The objective of the present prospective trial is to compare the efficacy of chlorhexidine varnish versus Fluoride varnish, on the *Mutans streptococci* (ms) counts, in the saliva of child patient, using Dentocult SM Strip *Mutans*.

Materials and Methods: This is a Prospective study done on sixty (60) patients, (4-5 years old) with fully erupted primary teeth, divided into four groups (n=15 in each): two fluoride varnishes groups [3% Sodium fluoride varnish (chemodent) and 1% Difluorsilane varnish (Fluor Protector)] , one chlorhexidine varnish group [1% chlorhexidine varnish (cervitec plus)] and one control group used fluoridate tooth paste. Saliva samples were collected on the strips from the Dentocult SM kit and after incubation, the presence of *Streptococcus mutans* was evaluated using the manufacturer's chart. The study groups were subjected to single varnishes application following which the samples were collected again after 24 hours, two weeks and one month.

Results: After 24 hours, there was no statistically significant difference between means % reduction in CFU scores of Chlorhexidine, Fluor protector and Fluoride Chemodent varnish groups. After 2 weeks, Fluor protector and Fluoride Chemodent varnish groups, showed the statistically significantly highest means % reduction than Chlorhexidine varnish and tooth brushing groups. After 1 month, there was no statistically significant difference between means % reduction in CFU scores of the four groups.

Conclusions: The results after two weeks showed that fluoride varnishes groups had a statistically significant reduction in the saliva *Streptococcus mutans* counts than the chlorhexidine varnish group and control group.

KEYWORDS: Dentocult SM strips, fluor protector, fluoride varnish, cervitec plus, chlorhexidine varnish, saliva, *Streptococcus mutans*.

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INTRODUCTION

Dental caries is the most common chronic disease in childhood, consisting in a severe public health problem for worldwide. It has a multifactorial etiology and developed upon the presence of the dental biofilm, which is responsible for mediating the demineralization of dental hard tissue. The interaction among three factors during a period of time promotes the development of caries. These factors are: cariogenic microorganisms (streptococcus mutans), fermentable substrate (such as sucrose) and a vulnerable host.¹ The cariogenic bacteria play a major role in initiation of early childhood caries (ECC), the earlier that a child's mouth is infected with Streptococcus mutans, the greater the risk for future caries development.² Children experiencing caries as infants or toddlers have a much greater probability of subsequent caries in both the primary and permanent dentitions.³ Saliva is considered the primary protection system in the oral cavity, it reduces friction on enamel, flushes bacteria and food away from the teeth, helps to neutralize the acid and replenishes the ions which remineralize the enamel. When this system is inadequate, for example if saliva is deficient, or if there are too many acid-producing bacteria due to poor oral hygiene or diet, the caries disease process can take hold and progress⁴, investigated the relationship between caries and the number of CFU/ml of streptococcus mutans and lactobacilli, salivary buffer capacity and salivary flow rate. 167 schoolchildren with primary dentition and Bacterial counts were evaluated by Dentocult and salivary buffer capacity was measured with Dentobuff. They found, positive association between streptococcus mutans and lactobacillus counts and the caries indices. Streptococcus mutans had been implicated as the principal bacterial component responsible for the initiation and progression of dental caries.⁵ Factors that may contribute to the virulence of Streptococcus mutans include acidogenicity, synthesis of extra polysaccharides that enhance adhesion to the tooth and increase the bulk to plaque,

synthesis of intracellular polysaccharides which allows acid production when sucrose is absent in diet, moreover they possess surface proteins called adhesions that bind to salivary glycoprotein on tooth surfaces which is essential to provide resistance to the flow of saliva.⁶ The Streptococcus mutans level and subsequent caries activity were often correlated, thus salivary Streptococcus mutans count was identified as a valuable test in predicting caries activity and in identifying individuals with high caries risk.⁷ Among the four methods of dental caries prevention, which are fluoridation, plaque control, diet and pit & fissure sealant, the most important remain fluoridation and diet.⁸ Preventive measures usually involve a combination of dietary counsels, oral hygiene measures and fluoride applications.⁹ Of all the professionally applied topical fluoride methods, fluoride varnish is the most practical for this age cohort; the varnish applications take less time (oral prophylaxis prior to application is not required), create less patient discomfort and achieve greater patient acceptability than fluoride gels, foams and rinses.¹⁰ Randomized controlled trial (RCT), of two years' duration, involving children with a mean age of 1.8 years, reported that the benefit of a combination of parental counseling plus varnish applied once, twice and three times a year reduced the mean dmfs by 53, 58 and 93%, respectively.¹¹ Jeevarathan et al.,2007¹² showed in their study marked reduction in the streptococcus mutans count in dental plaque after 24 hours of fluoride varnish application. Chlorhexidine is the most potent documented antimicrobial agent against Mutans streptococci (ms) and dental caries.¹³⁻¹⁴ It has been suggested, that chlorhexidine application in varnish form results in a longer-lasting suppression of Mutans streptococci compared with other forms of application.¹⁴⁻¹⁵ A split-mouth technique was followed in the treatment of 30 patients selected by stringent selection criteria, evaluating a single application of the test varnish on two randomly allotted quadrants along with a placebo on the other two quadrants. Chlorhexidine varnishes are capable

of reducing *Streptococcus mutans*. The side effects of chlorhexidine mouth rinses are not seen with this varnish.¹⁶

MATERIALS AND METHODS

Types of study

- The study trail will carry out on children selected from (Toyor Alsalam Association)*¹.
- Prospective study for sixty (60) patients with full erupted primary teeth, divided into four gropes:

- 1- **Group I** (n=15): Chlorhexidine varnish (cervitec plus) group which includes 1% chlorhexidine diacetate and 1% thymol as active antimicrobial ingredients, has been maintained
- 2- **Group II** (n=15) : Experimental (Fluor Protector) Fluoride varnish group. Fluor Protector contains 0.9% difluorsilane in a polyurethane varnish base with ethyl acetate and isoamylpropionate solvents.
- 3- **Group III** (n=15) : Proprietary (chemodent Sodium Fluoride 3%) Fluoride varnish group. A specially fluoride varnish prepared by Dr. Kamal El-Motayam, (Professor of Pediatric Dentistry and dental Public Health, Faculty of Oral and Dental Medicine, Cairo University) . Figure (1).
- 4- **Group IV** (n=15) : which only brushing their teeth by tooth paste contain fluoride (control group).

Types of participants (inclusion criteria)

- Patients aged from (4 to 5 years) of the same socio-economic level.
- Patients selection (boys or girls) .
- No history of intake of antibiotics for the past 3-4 weeks.
- No history of fluoride or chlorhexidine treatment for the past two weeks.

Types of interventions

The counts of *Streptococcus mutans* in saliva is determining using the simple chairside method (Dentocult SM Strip mutans orion Diagnostica, Espoo, Finland). Figure (2). For the baseline status of *Streptococcus mutans* .



Fig. (1) CHEMODENT Fluoride varnish.



Fig. (2) Dentocult ® SM strip Mutans.

Procedure of using Dentocult SM Strip mutans

- 1- Before sampling we using a needle or forceps to place a bacitracin disc in the selective culture broth inside the vials, about 15 min before sampling and then close the cap.
- 2- The vials should be taken to room temperature 1h before use and shaken gently .

* Toyor Alsalam Association, at the new city of Fustat – Cairo – Egypt, registered number(6838) at 2007 .

- 3- Specimen collection from saliva (using round-tipped strips): Use unstimulated saliva (without paraffin pellet) because the sampling collect from small children . Instruct the patient to swallow any excess saliva and then press the rough surface of the strip against the saliva remaining on the patient’s tongue. Avoid moving the strip on the tongue. Remove the strip through the patient’s gently closed lips.
 - 4- Shake the selective culture vial gently for even distribution of bacitracin.
 - 5- Place the strips, with the smooth surfaces clipped and attached to the cap, in the selective culture broth. The strips can also be incubated singly.
 - 6- Fill in the patient label and attach it to the vial.
 - 7- Incubate the vial in an upright position at 37 Co for 48 hours , with the cap one quarter of a turn open. Care must be taken to keep the caps one quarter of a turn open.
- The examination for the caries was accomplished visually by the same operator, this was performed according to the criteria of (dmf index):

dmf index : for primary teeth before shedding (pre-school age).

d = decayed tooth indicated for filling.

m = missing tooth due to caries.

f = filled tooth.

Interpretation of results

After incubation, the presence of mutans streptococci is evidenced by dark-blue to light-blue, raised colonies on the rough surface of the strip. Colonies suspended in the selective culture broth are excluded from the evaluation. Figure (3)

Mutans streptococci will adhere to the rough strip surface in proportion to their density in saliva. The density of mutans streptococci in saliva is obtained by comparing the colony density on the test strip with the model chart. Figure (4)



Fig. (3): Colonies on the strip.

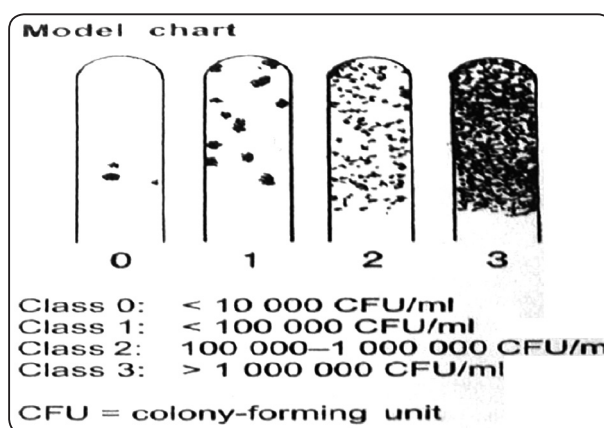


Fig. (4): Model chart of the test strips.

Limitations of the procedure

Epithelial cells detached from the tongue, lips and plaque will also turn blue during incubation. They can be differentiated from mutans growth by passing a gloved finger along the strip. Whereas epithelial cells on the strip surface will feel smooth, mutans streptococcal colonies will feel rough to the finger .

TABLE (1): Classes of colony-forming unit

Class 0	<10,000 CFU/ml (<i>ms</i> below detection level).
Class 1	<100,000 CFU/ml
Class 2	100,000-1000 000 CFU/ml
Class 3	>1000 000 CFU/ml
= CFU	colony-forming unit

- The results are interpreting by two independent interpreters who are blinding about the group division.
- Inspection of growth is done sideways against light or with a magnifying glass to look for raised colonies.
- Tooth brushing (control group) : depend on the mechanical effect of tooth brushing only by fluoride tooth paste, we firstly brushing their teeth by using the scrub technique, in which half circle strokes were applied for each jaw in up and down movement and then they were educated to use the tooth brush at home.
- After the collection of the saliva samples from all patients, the fluoride varnish, chlorhexidine varnish or tooth brushing are applying to the subjects of the study groups on the same day.

Procedure of application fluoride varnish or chlorhexidine varnish :

Step 1: Getting Ready

Supplies : fluoride varnish or chlorhexidine varnish, Gauze squares, Cotton rolls, Non-latex gloves.

Step 2: Child can sitting on the chair or stand in front of me .

Step 3 : Dry and clean the teeth Use the gauze squares.

Step 4 : A thin layer of varnishes are applying on all the tooth surfaces using a suitable brush.

Step 5: The cotton rolls were removed after one minute and the patient is asking not to rinse the mouth immediately and not to eat for 45 minutes and not to brush tonight.

- Second times after 24 h., third times After two weeks and forth times After one month , saliva samples are again collecting from the subjects of all the groups. These are also incubating for the same period as before and the same interpreters evaluated the results again.

RESULTS

(Table-2) shows there was no statistically significant difference between means dmf in the four groups.

(Table-3) shows the distribution of study groups according to sex and correlation with dmf. There was no statistically significant difference between means dmf in boys and girls of each groups.

The results of this study regard to Bacterial counts and related to period were :

1. Comparison between the groups in related to period (Table-4): There was no statistically significant difference between mean bacterial counts in the four groups through each period.
2. Changes in bacterial counts by time within each group:

In Chlorhexidine varnish group, there was a statistically significant decrease in CFU scores after 24 hours. After 2 weeks and after 1 month, there was non-statistically significant decrease in mean CFU scores (Table 5).

TABLE (2) The means, standard deviation (SD) values and results of Kruskal-Wallis test for comparison between dmf in the four groups

Chlorhexidine varnish		Fluor protector varnish		Fluoride Chemodent varnish		Tooth brushing		P-value
Mean	SD	Mean	SD	Mean	SD	Mean	SD	
4.7	2.7	4.5	2.3	4.7	3.2	4.3	1.9	0.997

TABLE (3) The means, standard deviation (SD) values and results of Mann-Whitney U test for comparison between CFU scores in boys and girls of tooth brushing group.

Group	Gender	Boy		Girl		P-value
		Mean	SD	Mean	SD	
Chlorhexidine varnish		5.1	3.1	4.3	2.4	0.480
Fluor protector varnish		4.7	2.6	4.4	2.3	0.725
Fluoride Chemodent varnish		4.8	2.5	4.7	4.1	0.814
Tooth brushing		4.9	1.6	3.6	2.2	0.218

TABLE (4) The means, standard deviation (SD) values and results of Kruskal-Wallis tests for comparison between CFU scores in the four groups.

Group Period	Chlorhexidine varnish		Fluor protector varnish		Fluoride Chemodent varnish		Tooth brushing		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1 st day	2.2	0.7	2.5	0.5	2.5	0.5	2.4	0.6	0.545
24 hours	1.4	0.9	1.7	0.7	1.9	0.9	2.2	0.5	0.099
2 weeks	2	0.8	1.9	0.5	2	0.8	2.2	0.7	0.494
1 month	2.1	0.6	2.1	0.5	2.3	0.6	2.3	0.6	0.700

TABLE (5)

Period	Mean difference	SD	P-value
1 st day – 24 hours	-0.8	0.8	0.006*
1 st day – 2 weeks	-0.2	0.6	0.180
1 st day – 1 month	-0.1	0.4	0.157

*: Significant at $P \leq 0.05$

In Fluor protector varnish group, there was a statistically significant decrease in CFU scores after 24 hours, after 2 weeks and after 1 month (Table 6).

TABLE (6)

Period	Mean difference	SD	P-value
1 st day – 24 hours	-0.8	0.9	0.010*
1 st day – 2 weeks	-0.6	0.6	0.007*
1 st day – 1 month	-0.3	0.5	0.025*

*: Significant at $P \leq 0.05$

In Fluoride Chemodent varnish group, there was a statistically significant decrease in CFU scores after 24 hours, after 2 weeks and after 1 month (Table 7).

TABLE (7)

Period	Mean difference	SD	P-value
1 st day – 24 hours	-0.7	0.7	0.008*
1 st day – 2 weeks	-0.5	0.6	0.011*
1 st day – 1 month	-0.3	0.5	0.046*

*: Significant at $P \leq 0.05$

In tooth brushing group, through all periods, there was non-statistically significant decrease in mean CFU scores (Table 8).

TABLE (8)

Period	Mean difference	SD	P-value
1 st day – 24 hours	-0.2	0.5	0.165
1 st day – 2 weeks	-0.2	0.6	0.180
1 st day – 1 month	-0.1	0.5	0.317

*: Significant at $P \leq 0.05$

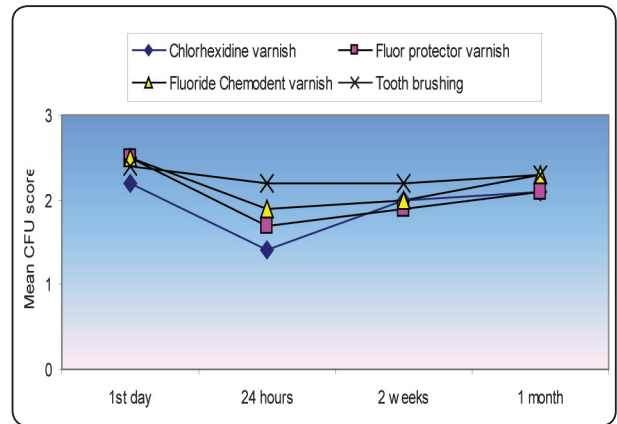


Fig. (5): Line chart representing changes by time in mean CFU scores in the four groups.

3. Comparison between % reduction in CFU scores: (Table 9).

The percentage reduction was calculated as :

$$\frac{\text{CFU score (1st day)} - \text{CFU score (after)}}{\text{CFU score (1st day)}} \times 100$$

After 24 hours, there was no statistically significant difference between means % reduction in CFU scores of Chlorhexidine, Fluor protector and Fluoride Chemodent varnish groups, but there was statistically significant difference between the three groups and tooth brushing group. After 2 weeks, there was no statistically significant difference between means % reduction in CFU scores of Fluor protector and Fluoride Chemodent varnish groups, but there was statistically significant difference between the two groups and Chlorhexidine varnish and tooth brushing groups. After 1 month, there was no statistically significant difference between means % reduction in CFU scores of the four groups.

There were statistically significant for the four groups after 24 hours , other than 2 weeks and one month.

TABLE (9) The means, standard deviation (SD) values and results of Kruskal-Wallis tests for comparison between % reduction in CFU scores in the four groups

Group	Chlorhexidine varnish		Fluor protector varnish		Fluoride Chemodent varnish		Tooth brushing		P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
% reduction in CFU after 24 hours	37.8 ^a	39.1	30 ^a	33.5	27.8 ^a	34.3	6.7 ^b	16.3	0.031*
% reduction in CFU after 2 weeks	10 ^b	32	22.2 ^a	23.3	21.1 ^a	28.5	6.7 ^b	23.4	0.046*
% reduction in CFU after 1 month	4.4	11.7	12.2	18.3	10	17.6	3.3	20.1	0.504

*: Significant at $P \leq 0.05$, Means with different letters are statistically significantly different according to Mann-Whitney U test.

DISCUSSION

The age of children who participated in this study ranged from 4-5 years and this is in accordance with Adair *et al.*, 2001¹⁷ who stated that, the early childhood caries (ECC) was considered to be the most prevalent infectious disease of pre-school children with ages ranging between 3-6 years. The approval consent and the selection of children was done from one association (Toyor Alsalam) at the new city of Fustat – cairo – Egypt, to ensure comparable socioeconomic and feeding habits of all participants. Pooled saliva samples were used to determine levels of Streptococcus mutans because the Levels of salivary mutans streptococci are potential good predictors of caries experience among 4–5 years-old children,¹⁸ and the more areas of dentition affected by dental caries are more affected by mutans streptococci, and is the most higher bacterial count in the saliva of small children.¹⁹ There is a correlation between the occurrence of mutans streptococci in plaque and that in saliva, if saliva contains high bacteria counts, plaque does too. High counts in saliva correlates to $> 10^3$ CFU mutans streptococci in plaque.²⁰ The saliva counts are considered to be a reasonable indicator of the entire dentition's total microbial

load , and the number of mutans streptococci or lactobacilli in a total dental plaque sample does not explain the variation in caries better than the numbers in stimulated saliva.²¹⁻²² In this study we studied the streptococcus mutans in the saliva because there is close association between salivary mutans streptococci and caries incidence in preschool children,²² and the Streptococcus mutans level and subsequent caries activity were often correlated, thus salivary Streptococcus mutans count was identified as a valuable test in predicting caries activity and in identifying individuals with high caries risk.⁷ Of all microorganisms we select in this study only mutans streptococci (MS) because it is the most closely associated with the development of dental caries disease.¹⁻⁵ According to Shi *et al.*, 2003,²³ Dentocult SM is the best test for the diagnosis of the presence of caries and its prognosis with a high assessment and a high statistical significance. The advantages of this test include that it is chairside assuring greater patient compliance especially for young subjects, it needs minimal armamentarium, it is less time-consuming and it facilitates sample collection. Follow up samples were taken as it has been stated that the best cariostatic effect of antibacterial regimens is

achieved when the outcome of the treatment is verified with follow up samples and the prospective study design enabled us to monitor the outcome of the antibacterial regimens regularly during the study period.²⁴ All the variables at baseline were compared in all groups. The non statistically significant difference between the mean dmf and the mean streptococcus mutans counts ensures the similarity of the variables between the groups at baseline. Therefore, the impact on the results due to the imbalance in variables between the groups was probably negligible. There was no statistically significant difference in the mean score of streptococcus mutans count in relation to sex in all groups during the study and that was in accordance with previous studies.²⁵⁻²⁶⁻²⁷ After varnish application in all groups, there was a statistically significant difference between the pre and post treatment bacterial scores [Table 9]. In group IV, which only brushing their teeth by tooth paste contain fluoride (control group) : The result showed that there was non-statistically significant decrease in mean CFU scores after 24 hours, after 2 weeks and after 1 month. This result was in agreement with *Petersson et al.,1991*; *Yoshihara et al.,2001*,²⁸⁻²⁹ there are no difference in the level of mutans streptococci between subjects using or not using fluoridated toothpaste. In group I, Chlorhexidine varnish (cervitec plus) group : The result showed that there was a statistically significant decrease in mean CFU scores after 24 hours ($p=0.006$) . After 2 weeks ($p=0.180$) and after 1 month ($p=0.157$) , there was non-statistically significant decrease in mean CFU scores. Chlorhexidine in high concentrations, i.e. 100 ppm is capable of destroying the cell membranes of bacteria and thus has a bactericidal effect. A bacteriostatic effect is achieved at only 0.11 ppm chlorhexidine.³⁰ These results agreed with *Wallman and Birkhed, 2000*,³¹ who showed that 1% chlorhexidine varnish was applied twice, with a 3–4 days interval between applications. A significant lowering of dental biofilm mutans streptococci (MS) was found 1 week after the end of applications.

Evaluation of the treatment 1 and 2 months later showed a progressive increase in mutans streptococci (MS) levels; during the 1-month post treatment evaluation, however, values were still significantly lower than those recorded prior to treatment. After 2 weeks and after 1 month, there was non-statistically significant decrease in mean CFU scores because with low concentrations of chlorhexidine mutans streptococci (MS) may not be killed effectively and proliferate and return to their original numbers within a few weeks.³² And *Petersson et al.,1991*,²⁸ examined interproximal plaque after treatment with a lowly concentrated chlorhexidine varnish (Cervitec) they found a gradual return to pretreatment values at their 1- and 3-month examination. In this study only we apply single application of (Cervitec) chlorhexidine varnish, and for chlorhexidine varnishes with low concentration like (Cervitec), repeated application and a longer retention time are required.³³ Also, *Yucel-Lindberg et al.,1999*,³⁴ found that the findings between placebo and chlorhexidine varnish treatments on days 3, 8, and 30 were not significant; they attributed this result to the cross over effect of the test varnish. *Plotzitz et al.,2005*,²⁷ suggested that in patients with cariogenic dietary habits and poor oral hygiene, chlorhexidine is not effective in reducing mutans streptococci (MS) . *Zhang et al.,2006*,³⁵ suggested that the amount of chlorhexidine that is released from varnish is too low to be effective on other sites of the dentition. *Ribeiro et al.,2007*,³⁶ which compare different formulations of chlorhexidine at reducing the levels of MS in the mouth, found no difference in the decrease in MS levels observed in the split-mouth trials compared to other studies with control groups. On the contrary , *Du et al.,2006*,³⁷ found that 40% chlorhexidine varnish in 4- to 5-year-old children with low background exposure to fluoride. Although the overall 2-year caries increment in primary molars was quite low, a statistically significant reduction in the caries increment in dentine was reported for children in the chlorhexidine varnish group compared to children in the placebo group.

The results suggested a 37.3% reduction in caries increment over 2 years. But the participants are children (4-5 years) and may be not accepted to the treatment with the highly concentrated varnish (40% chlorhexidine varnish) due to displayed side effects like desquamation of mucosa and taste disturbances in some subjects. These effects disappeared 2-3 days after application.³¹ The rationale for using chlorhexidine to prevent caries is based on its ability to effectively suppress MS. The duration of suppression of MS is variable and may be influenced by the chlorhexidine concentration of the varnish.³⁶ Evidence regarding the effectiveness of chlorhexidine varnish for preventing caries is inconclusive. Further well-conducted randomized trials are required before chlorhexidine varnish can be recommended for caries prevention.³⁸ In group II Experimental (Fluor Protector) fluoride varnish & group III Proprietary (Chemodent) fluoride varnish group : The result showed that there was a statistically significant decrease in mean CFU scores after 24 hours (group II $p=0.010$) (group III $p=0.008$), after 2 weeks (group II $p=0.007$) (group III $p=0.011$) and after 1 month (group II $p=0.025$) (group III $p=0.046$). Fluoride from topical sources is converted partially to hydrogen fluoride (HF) by the acid that the bacteria produce and diffuses into the cell, thereby inhibiting essential enzyme activity.³⁹ These Results agreed with *Castillo et al., 2001*,⁴⁰ who showed that sodium fluoride varnish released fluoride for 19 weeks after application to primary molars enamel stored in buffered calcium phosphate solution. Also, *Jeevarathan et al., 2007*,¹² found a statistically significant difference between the pre- and post- treatment bacterial plaque scores 24 hours after varnish application. Hence, the reduction of bacterial counts it could be due to the high concentration of fluoride from Fluor Protector (1000 ppm) that might have entered the bacterial cell and resulted in the inhibition of various cellular processes. *Ekenbäck et al., 2000*,⁴¹ found that hydroxyapatite crystals pretreated with Fluor Protector and sodium fluoride showed a statistically

significant reduction in lactic acid formation in *Streptococcus mutans*. All fluoride varnishes (Fluor Protector : 1% Difluorsilano) and (5% sodium fluoride) increased the fluoride concentration in plaque compared with baseline.⁴² All fluoride varnishes (Fluor Protector : 1% Difluorsilano) and (5% sodium fluoride) significantly reduced the lactate production compared to untreated controls or placebo discs.⁴³ *Seppä et al., 1982*,⁴⁴ found that the amount of fluoride deposited in teeth is high after the use of Fluoride varnishes. High levels of fluoride could eliminate susceptible micro-organisms and modify the plaque ecosystem. *Azarpazhooch and Main, 2008*,⁴⁵ shown no difference in efficacy with multiple applications of varnish within a short period (e.g., 3 applications within 2 weeks). The authors concluded that school-based application of fluoride varnish every 6 months is an excellent way of preventing a proximal caries in Children living in areas with medium and high caries risk. The American Academy of Pediatric Dentistry recommends professional use of fluoride varnish to prevent or reverse the demineralization of dental enamel in children with a moderate to high risk of dental caries. American Academy of Pediatric Dentistry, 2009 (*AAPD, 2009*).⁴⁶ By comparing the results of (group I) and (group II, III), there was no statistically significant difference between the mean scores of streptococcus mutans count in all groups before varnish application and after 24 hours. However a statistically significant difference was noted on comparing mean scores of streptococcus mutans count two weeks and one month after varnishes application. *Petersson et al., 2000*,⁴⁷ compared the effectiveness of 3-monthly 1% chlorhexidine-thymol varnish applications with 3-monthly 0.1% fluoride varnish applications for preventing a proximal caries in a group of Swedish adolescents with background exposure to topical fluoride applications. The caries increment measured radio graphically after 3 years was mostly in enamel, the authors reported that the difference was not statistically significant. *James et al., 2010*,³⁸

Evidence of the comparative effectiveness of chlorhexidine varnish and fluoride varnish comes from randomized trial *Petersson et al., 2000*⁴⁷ is equivocal. The variables may be influenced due to study design, concentration of varnish used, application frequency, type of control group and dentition to which the varnish was applied.

CONCLUSION

1. Fluoride varnish is a highly efficient caries preventive vehicle for preschool-aged children as it is quick, easy to apply and is less likely to be swallowed by young children (no adverse toxic, sensitizing or irritating effects could be observed).
2. The enhanced slow release, sustained action and have significant long term effect on the streptococcus mutans more than two weeks of fluoride varnish application.
3. The single application of 1% chlorhexidine varnish to the primary teeth was capable of significantly reducing the streptococcus mutans after the first 24 hours but had no after two weeks and the end of one month.
4. Children in this study readily accepted 1% chlorhexidine varnish with no complaints of taste, discomfort or discoloration.

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